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v

PATHOLOGICAL CHANGES ACCOMPANYING INJECTIONS OF AN ACTIVE DEPOSIT OF RADIUM EMANATION

I. INTRAVENOUS AND SUBCUTANEOUS INJECTIONS IN THE WHITE RAT

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From the Huntington Fund for Cancer Research, Memorial Hospital, New York City

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Very little is known concerning the changes that occur in living tissue following the injection of solutions of an "active deposit" of radium emanation. This investigation was undertaken to determine what changes occur in the principal organs of the animal body following such a treatment, and to afford data that might act as a guide in the treatment of certain types of cancer, in which the solution method of radium therapy appeared particularly adapted. Since the main facts of this investigation became apparent, cases of leukemia and lymphosarcoma have been treated by the methods employed in these experiments. In these diseases the lesions were so widely disseminated as to suggest treatment by the method of intravenous radium injection.

The scope of the investigation may be divided into two parts, one dealing with experiments concerning intravenous, and the other with subcutaneous injections of the "active deposit" of radium emanation.

APPARATUS AND METHODS

At the Memorial Hospital the Duane type of radium emanation apparatus is used. It was devised by Doctor William Duane, and adequately described in The Boston Medical and Surgical Journal (1).

Instead of collecting the radium emanation by compressing it in small glass tubes, this being the usual method, the emanation for these experiments was collected in the form of what is called an "active deposit" upon common salt. A small quantity of salt was placed in the upper glass bulb, attached to a larger bulb as indicated in figure 1, and the bulbs were then sealed to the end of a radium emanation purifying apparatus. Then a considerable quantity of emanation, in the form of a gas, was forced into the bulb containing the salt. This was done by first creating a vacuum, and later forcing the purified emanation ahead of a column of mercury, until all the emanation was contained in the small bulb in which the salt had been placed. After about three hours the salt had received the maximum amount of radio-active deposit. The glass bulb was then cut from the apparatus, and the salt, containing the active deposit of radium, was dissolved in sufficient distilled water to bring the solution to the strength of a physiological salt solution. The liquid was drawn into a 2 cc. Luer syringe, which was covered by a lead shield as a protection for the fingers of the experimenter, and the syringe, needle, and solution were measured in an accurate electrometer. The activated solution exhibited all the known

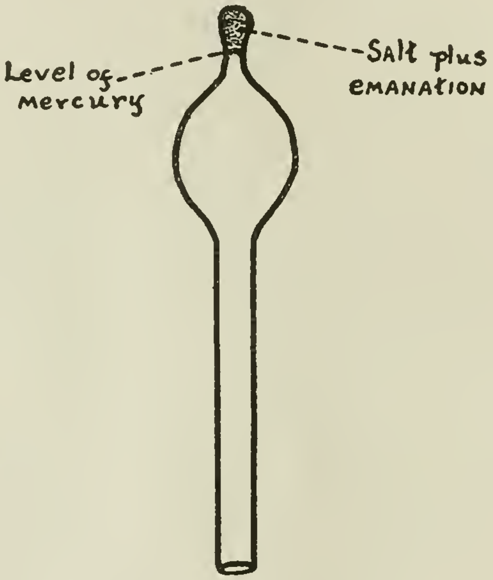

Fig. 1 Apparatus used in collecting active deposit of radium.

phenomena of radium metal itself. The strength of the solution was determined in millicurie units. In this manner a considerable quantity of active radium substance was obtained in a relatively small amount of liquid. For example, the usual amount collected was about 150 mc. in 2 cc .of solution.

This method of collecting radium deposit was found to be very economical. Only a little of the main supply of the hospital's emanation was actually used, since only a small part, 2 to 3 per cent, of the emanation had actually been given off as a deposit on the salt, and after the three hours exposure the remaining emanation was removed and collected in glass tubes for routine therapeutic use.

In the solution used in these experiments the radiation consisted of alpha, beta, and gamma rays, but the greatest physiological effects produced in the tissues were probably the result of alpha ray activity. (For a further consideration of the physical properties of radium emanation see references (2), (3) and (4).)

Following the well known physical phenomenon, the radioactivity, after reaching its maximum strength in three hours, decayed at a uniform rate, and so it was necessary to dissolve the salt, measure the syringe and needle, and inject the solution into the animals with as little loss of time as possible. However, in all cases a larger amount of radium was collected than was needed at one time, so that one might take advantage of the radium decay by waiting until the solution had reached a desired dosage before the injections were made. If preparations are made in advance for quickly injecting the solution, only a relatively small amount of the radio-activity is deposited upon the walls of the syringe and the needle. After the injection is made the empty syringe and needle are at once measured in an electrometer, so that the amount of radio-activity left in the apparatus may be determined. When this is subtracted from the previous figure, obtained for the syringe plus the solution, the amount of radium that was actually contained in the solution may be calculated. After a little experience it is possible to approximate very nearly a required dose. All the doses were given in millicurie units of "active deposit" of radium emanation as described above, and if reference is later made to the injected solution as, radio-active substance, activated solution, etc., be it understood that these terms are used interchangeably with "active deposit."

Only healthy, three-quarters to full grown, rats were used in this investigation. They varied in weight from 200 to 250 grams. The injections were made under light ether anesthesia. Caudal vein injections were made in the intravenous series, while in the subcutaneous groups the solutions were deposited under the skin in the right shoulder region.

Some of the animals were killed by the experimenter for histological study. These animals were put under ether anesthesia and the organs quickly removed and placed in fixative.

EXPERIMENTAL RESULTS

Series A. Intravenous injections

In this series twelve animals were used, and caudal injections were given in doses ranging from 2.6 mc. to as high as 135 mc. each.

Table 1 gives in the first two columns, the serial number for each animal, and the dose employed in millicuries. In the third column is indicated the time when the animals died, apparently as a result of the treatment. The last column indicates the day on which the animals that received the smaller doses were killed by the experimenter for histological study. We find in table 1, that doses ranging from the smallest, 2.6 mc., to 10.6 mc., were not sufficient to cause the death of the animals within a month's time after the injection. From this group of animals that survived the treatment for a considerable period, one was killed on the thirty-fifth and another on the thirty-seventh day after treatment. Two were killed on the forty-fourth and two on the forty-fifth day, and finally one was killed on the seventy-eighth day after treatment. The table indicates however, that an increase from 10.6 mc. to 11.2 mc. proved fatal in the case of animal VIII. This animal died within two and a half days after treatment. Likewise, all the doses above 11.2 mc. resulted in immediate, acute effects. Animal IX, after receiving an intravenous injection of 19.8 mc., was so ill at the end of four days that it would soon have died as a result of the treatment, had it not been killed by the experimenter in order that its organs might be preserved in the most favorable condition for histological study. Animal X received a dose of 27.8 mc., and died at the end of three days. In like manner animal XI, that received 29.6 mc., after showing very severe symptoms, died at the end of the third day. Animal XII, after receiving 135 mc., the largest dose of radium that was used in any one instance, died a few hours after the injection.

TABLE 1

Series A. Results for the intravenous injections of solutions of "the active deposit" of radium emanation

ANIMAL NUMBER	NUMBER OF MILLICURIES OF RADIUM EMANATION	DAY ON WHICH ANIMAL DIED AS A RESULT OF THE TREATMENT	THE DAY FOLLOWING THE TREATMENT ON WHICH THE ANIMAL WAS KILLED FOR AUTOPSY
I	2.6		45
II	2.9		78
III	4.3		45
IV	5.7		44
V	7.1		35
VI	8.8		37
VII	10.6		44
VIII	11.2	2.5	
IX	19.8	*	
X	27.8	3	
XI	29.6	3	
XII	135.0	†	

* Was very ill at the end of the fourth day and was then killed.

† Died a few hours after the treatment, between 5 p.m. and 9 a.m.

The experimental data that follow give the histological reports for the twelve animals that received radium treatment by the intravenous method. The organs showing the greatest pathological changes were, the liver, the lungs, the kidneys, and the spleen. The bone marrow and adrenals were considerably altered in certain cases, and changes occurring in the vascular supply of the brain were noted. No data were obtained for the changes that occurred in the blood following the treatments as it was decided to test this point on a larger type of animal. This is now being done, and the results will be published at a later time.

The experiments are presented according to the size of the dose employed. From the standpoint of the general physiological reactions, animals I to VII inclusive may be said to show more or less chronic effects, while animals VIII to XII, that received the larger doses, exhibited decidedly acute reactions. Animal III, for example, was treated on June 10, 1918, with an injection of 4.3 mc., and was killed by the experimenter forty-five days later. This was one of the smaller doses, for, as may be seen in table 1, animal VII was treated with as large a dose as 10.6 mc. and still lived until it was killed forty-four days later. And yet the pathological findings for animal III showed an intense congestion of the liver, with severe acute degeneration of a fatty granular nature. The kidneys were also intensely congested and hemorrhages occurred in the glomeruli, cortex, and medulla. A condition of purulent bronchitis and bronchopneumonia was found in the lungs, while the spleen was intensely congested and hemorrhagic, with reduction in size of the follicles.

It was found, that even with a still smaller dose, such as 2.6 mc., which was given to animal I, there was produced in the kidneys an intense acute congestion, edema of the lungs, and also reduction in size of the lymph follicles in the spleen. In the last two organs, however, the small dose of radium produced no hemorrhages, and it is well to note here that with larger doses congestion associated with hemorrhages occurred in these organs almost constantly.

Animal IV was treated with 5.7 mc. of radium, and, although the liver of this animal showed some congestion, still this condition was less marked than was found for the larger doses. There was but little congestion in the kidneys, and the lungs were in a fairly good condition. In this case the spleen appeared considerably affected. It was intensely congested, and this condition was almost as pronounced as that which occurred in the spleen of animals that had received very large doses.

With a somewhat larger dose of radium, 7.1 mc., animal V presented changes in the liver similar to those of animal IV; but in the case of animal V, the kidneys and lungs showed intense congestion, while in the spleen no appreciable congestion was found. In the case of animal VI, that received a somewhat larger dose, 8.8 mc., and was killed by the experimenter thirty-seven days after the treatment, the principal conditions noted in the organs were: a cloudy swelling of the liver cells, with advanced parenchymatous hepatitis; congestion of the kidneys, adrenals, spleen, and lungs, and also in the last named organ a perivascular edema.

The largest dose of radium that was survived for a considerable length of time, occurred in animal VII. This animal received 10.6 mc. and was killed, for purposes of autopsy, on the forty-fourth day. The liver was congested, but the condition in this case was not as marked as in the animals that received the still larger doses. The kidneys were in a state of severe granular degeneration, with intense congestion, while the swelling of the tufts of the glomeruli filled the capsules completely, but there was no cell necrosis. The lung lesion was an acute bronchitis, associated with areas of bronchopneumonia. The spleen showed intense hyperplasia of the lymph follicles, and some congestion.

As we go from animal VII to animal VIII, although the increase in dose was only 0.6 mc., we find in the latter case that the animal died two and a half days after its treatment. The dose in this case was 11.2 mc. and following it we find the occurrence of more active, acute conditions. The liver contained focal areas of necrosis, usually near the blood vessels and associated with hemorrhage. The kidneys were congested and hemorrhagic, and the lungs showed intense congestion, with hemorrhagic pneumonia. There was an edematous condition about the blood vessels and the bronchi. However, the spleen and also the brain, showed but relatively little congestion. Plate 1 shows the conditions that resulted in the kidney of this animal.

Following an injection of 19.8 mc. of radium, animal IX soon became very ill. It exhibited all the signs of an acute enteritis, and it was killed by the experimenter on the fourth day after its treatment. The liver of this animal was intensely congested, and the kidneys showed hemorrhagic nephritis, with dilatation of the large blood vessels. The lungs in this case were also congested.

Animal X received an injection of 27.8 mc., and died as a result of the treatment three days later. As in the preceding case, the animal also exhibited severe symptoms of an intestinal disturbance. The histological data showed an intensely congested liver, especially of the capillaries. There was perivascular edema of the large blood vessels of the portal canals, and in some cases there appeared to be a beginning thrombosis and fibrinous deposit in the blood vessels. In the kidneys a severe, acute degeneration had taken place, which was associated with an intense congestion, involving to a marked extent, the glomeruli and the cortical tubules. The splenic follicles were prominent. The splenic pulp, the brain, and the lungs were intensely congested. In the lungs a partial destruction of the large veins was noted.

On January 10 animal XI received 29.6 mc. of radium. On January 12 it appeared fairly normal, but on the next day it became very ill and died during the following night. The kidneys were congested, and a granular degeneration was present in the cells of the tubules. In some places this degeneration almost amounted to necrosis. In the lungs there was extreme venous and capillary congestion. This marked congestion had also extended to the spleen. The sinuses of that organ were choked with large phagocytic cells and old blood pigment, indicating a considerable red blood cell destruction. The follicles in the spleen were reduced in size. The bone marrow of the sternum was extremely congested. Few marrow cells remained, and in some places there were none at all. The marrow spaces were occupied by a deposit of diffuse blood. The fat cells were indistinct, and many appeared to be broken up into globules of various sizes.

The largest dose of radium administered at one time was given to animal XII. This animal received an intravenous injection of 135 mc. It died during the night of the same day, between 5 p.m. and 9 a.m. Unfortunately the liver and spleen were destroyed by its cage mates, but satisfactory sections were obtained from the kidneys, adrenals, lungs, and bone marrow. The kidneys showed diffuse congestion, and there was enormous congestion in the adrenals. In the lungs the alveoli were filled with red blood cells. There was a marked perivascular and peribronchial edema, and there were also evidences of an interstitial bronchitis, with a marked desquamation of the epithelial cells of the bronchi.

A recapitulation and discussion of these results will be deferred until the following data for the second series, series B, are given.

TABLE 2

Series B. Results for the subcutaneous injections of the "active deposit" of radium emanation

ANIMAL NUMBER	NUMBER OF MILLICURIES OF RADIUM EMANATION	DAY ON WHICH ANIMAL DIED AS A RESULT OF THE TREATMENT	THE DAY FOLLOWING THE TREATMENT ON WHICH THE ANIMAL WAS KILLED FOR AUTOPSY
I	3.5		64
II	3.5		64
III	7.0		64
IV	9.1		6
V	9.1		4
VI	17.0	5	
VII	17.0	4	
VIII	19.0		7
IX	19.0	6	
X	20.5	5	

SERIES B. SUBCUTANEOUS INJECTIONS

In table 2 are given the results obtained from the series of subcutaneous injections. These injections were made under the skin in the right shoulder region. The method of arranging the data is the same as in the first table. Comparing the two sets of results we find them similar in regard to the lethal effects that were produced in the animals. Here it is seen that doses up to about 10 mc. are not fatal. In this series a subcutaneous injection of 17 mc. killed an animal in five days. It is possible that the destructive effects produced from subcutaneous injections are less severe than in the case of the intravenous injections. The data suggest this conclusion, but are not sufficient to be conclusive. This point will be discussed later in the report.

Two animals received subcutaneous injections of 3.5 mc. each, and lived until they were killed by the experimenter sixty-four days after treatment. Another animal, that received 7.0 mc., was killed in the same manner on the sixty-fourth day. Two animals, each receiving 9.1 mc., were killed on the sixth and fourth day respectively. Two animals received doses of 17.0 mc. each, and they died, apparently as a result of the treatment, one on the fifth, and the other on the fourth day following the injections. Two more animals were injected and each received 19.0 mc. One died at the end of six days, the other was in a fairly good condition when killed by the experimenter. This animal, no. VIII of series B, was apparently exceptional in its resistance to the lethal effects of radium. Its organs suffered definite lesions, however, which will be described later. The tenth, and last animal of the series, was killed in five days by a dose of 20.5 mc. It exhibited symptoms of severe intestinal disturbance.

In the data that follow for the series of subcutaneous injections, it will be noted that, as in the case of the intravenous injections, the liver and kidney suffer largely as a result of exposure to radium emanation. However, probably due to the relatively slower rate of diffusion throughout the body in the case of the subcutaneous method, the lungs in this series are very much less affected. Only three out of ten animals that received subcutaneous injections gave definite lung lesions, while the lungs of the remaining seven animals were apparently normal. This condition held even after comparatively large doses.

It was also noted that the local reaction of the subcutaneous tissues in the region of the point of injection was slight. This is probably due to the fairly rapid manner in which the solution was diffused in the tissues of the shoulder region. In none of the cases was necrosis produced. In some of the early caudal vein injections, however, a small part of the radium was deposited outside the blood vessel, and in such cases a small necrotic area was produced at the point of injection. This condition resulted because the diffusion was slow, and the radio-activity was deposited locally.

Animals I and II received subcutaneous injections of radium amounting to 3.5 mc. each. They were killed by the experimenter sixty-four days after the treatment. The liver in both cases showed congestion and fatty degeneration, with the presence of many giant cells, and numerous hyperchromatic nuclei. In each case the lungs were normal, while the bone marrow and spleen of animal I showed no definite lesions, yet the kidneys of this animal were much congested. The renal tubules appeared normal, but there was hyperchromatism of the cells of the glomeruli. In animal II the renal tubules were dilated and filled with coagulum, while in the cells there was a granular degeneration. The bone marrow was congested, but otherwise was little altered, while the spleen was severely congested and contained prominent follicles.

A single subject, animal III, was injected with 7.0 mc., and was killed sixty-four days later. In this animal the liver showed the greatest changes, exhibiting a very marked fatty degeneration, with the nuclei of many cells much enlarged and hyperchromatic. The kidney was normal, but for a certain amount of capillary congestion. The lungs, bone marrow, and spleen were normal, except that the spleen was small, and in the bone marrow there was some dilatation of the sinuses.

Two animals, nos. IV and V, were each given a subcutaneous injection of 9.1 mc. of radium. The former was killed on the sixth day and the latter on the fourth day following. Animal V showed but slight degeneration of the liver, but there was a multiplication of the nuclei of the cells of that organ. The liver of animal IV, however, showed fatty degeneration and was severely congested. The kidneys in both animals were congested, but showed only a slight degeneration. The lungs of animal V were normal, while in animal IV, bronchopneumonia and catarrhal bronchitis were present, and in the latter animal there was congestion of the bone marrow, and but few foci of the marrow cells remained.

Animals VI and VII were each given subcutaneous injections amounting to 17.0 mc. One died four days later, and the other five. Before death each exhibited symptoms of severe intestinal disturbances. Unfortunately the histological data are lacking, and for this reason approximately the same doses were repeated for two other animals, nos. VIII and IX. These latter each received a dose of 19.0 mc. Animal IX died on the sixth day, but animal VIII proved particularly resistant and lived until it was killed on the seventh day. Although animal VIII lived for a week after its treatment, and was apparently in a fairly good condition before it died, still its organs proved to have suffered profound pathological changes. A severe granular and fatty degeneration occurred in the liver which was associated with much congestion, and the production of cells with large nuclei. In the kidneys there was congestion and granular degeneration, while the lungs contained a slight bronchopneumonia, with perivascular edema. There was not much congestion in the spleen, but an increase in size of the malpighian bodies was noted.

The following results were obtained for animal IX. Congestion and acute degeneration were present in the liver and kidneys, while in the lungs there was a marked bronchopneumonia. In the spleen great congestion had occurred, with damage to the splenic cells. The malpighian bodies were small, and the bone marrow was largely replaced by blood.

The largest subcutaneous injection was given to animal X. The animal received 20.5 mc., and died after five days. There was found in the liver a very severe congestion and degeneration of the hepatic cells, with the formation of many giant cells, with large nuclei, which were homogenous and hyperchromatic. The renal tubules were dilated, and a granular degeneration and erosion of the cells had taken place. The lungs were normal, but the bone marrow was replaced by blood, no marrow cells being retained. The conditions occurring in the liver of this animal are shown in plate 2.

SERIES C. CONTROL GROUP

A large series of sections was used as a control to these experiments. These sections were taken from normal untreated animals, killed in the same manner as the treated individuals, and also from animals injected intravenously and subcutaneously with salt not exposed to radium. A third control group was composed of animals injected with salt that had been exposed to a very large amount of radium emanation, and was then allowed to "decay," until the active deposit had lost its radio-activity. These animals came from the same stock that was used in series A and B, and were of about the same size, weight, etc., as the animals of the experimental groups.

The animals treated with ordinary salt, and those treated with salt that had been exposed to a large amount of radium emanation and then allowed to "decay," showed absolutely no symptoms of enteritis and appeared to be perfectly normal, as regards both their physical condition and the histological study of their organs.

DISCUSSION OF RESULTS

The results of this investigation show that injections of an active deposit of radium emanation, applied by the intravenous or the subcutaneous methods, bring about very definite changes in the animal body. It has also been found that sublethal doses, although they permit the animal to live for a fairly long time, result in pathological changes in the organs of a more or less chronic nature. It is noteworthy, in this regard, to refer to the changes that occur in the liver, especially those resulting from small doses of radium injected subcutaneously. Here we find a fatty degeneration, which, in the animals of this series, was very pronounced and frequent and was found to be present a long time after treatment. This condition was associated with the appearance of many giant cells and large nuclei, probably as a result of a regenerative process. It is interesting to note at this point that Mills (5) reported in 1910, a similar condition resulting from external applications of radium. He found that by exposing a series of mice to gamma rays, and using for thirty minutes over the region of the liver, what amounted to about 25 mgm. of radium bromide, "a transient change in the liver cells, somewhat resembling a cloudy swelling," took place. In the present investigation, as previously stated, the changes in the tissues are probably due mainly to alpha ray activity, and so it would appear that the type of degeneration referred to above may result from one or from a combination of the three types of radiation given off by the radium.

Following comparatively large doses of radium the animals died in from a few hours to six days. The accompanying lesions were severe congestion, frequently associated with diffuse hemorrhages, in the liver, kidney, lung, spleen, and bone marrow. In the case of intravenous injections the lungs were found to be severely affected. The large doses of radium produced a proliferation and desquamation of the epithelial cells of the bronchi, and there was also an apparent rupture of the blood vessels themselves, so that the air spaces were filled with red blood cells. This condition was associated with marked edema about the blood vessels and the bronchi. A similar vascular response for the blood vessels of the skin, was observed by Thies (6), for, after exposing the skin of guinea-pigs for six hours to 20 mgm. of radium bromide, he noted a dilatation of the blood vessels, and the presence of capillary hemorrhages.

This investigation shows that in the kidneys the most frequent condition following, or accompanying congestion, was a granular degeneration and erosion of the renal cells.

The changes in the bone marrow resulted in its replacement by blood. In many cases it was found that following a radium injection no marrow cells remained. This observation confirms the work of Thies, obtained by irradiating white mice in toto.

Congestion of the spleen was found to be the most constant feature resulting from radium treatment. This was also found to be associated, in some cases, with hemorrhage and a destruction of red blood cells.

In certain cases, following fairly large doses of radium, the blood vessels of the brain were found to be intensely congested.

The small vessels were dilated and gorged with blood. This observation is in line with that of Danysz (7), who reported the presence of hemorrhages in the brain and spinal cord of mice exposed to radium.

It seems fairly evident from a comparison of the results of this investigation with those already obtained by other investigators, that the changes that occur in the animal tissues following an injection of a radio-active solution, either in the blood stream directly, or indirectly by the subcutaneous method, are apparently similar to the changes that follow the external application of radiations from radium bromide itself.

There have been some data accumulated concerning the elimination of radium after its injection in the animal body, and they are mentioned at this point as they probably have some bearing on the interpretation of the results of this study. To quote from Meyer (8), 1906–1907, who obtained his results by using solutions of radium bromide, "The liver, lungs, and kidneys appear to be among the first organs to show the presence of radium after its intravenous injection. The ultimate fate of radium introduced subcutaneously, intraperitoneally, or per os is not materially different from that of radium introduced intravenously."

Berg and Welker (9) reported, 1905–1906, that "after subcutaneous injections, radium (bromid), like barium, calcium, and similar elements, is eliminated per rectum. The intestine seems to be the main channel of radium excretion."

Salant and Meyer (10), 1907–1908, found that "the elimination of radium takes place chiefly through the liver, the kidneys and the small intestine, and to a lesser extent also, through the large intestine in some of the herbivora."

From some unpublished work of the present writer, it was found that if shortly following an intravenous injection of the "active deposit" of radium emanation, prepared as described in this article, the blood vessels of the large viscera were ligated, and the organs, plus the blood they contained, were then tested for their radio-activity, the liver showed the presence of the greatest amount of this activity. The activity found in the two kidneys together was about a third the amount found in the liver. The stomach and intestinal tract contained slightly more radium than the kidneys, and the lungs slightly less than half the amount detected in the latter, while in the spleen there was a little less radium than was detected in the lungs. This work was performed on the rabbit, and will later be reported in more detail.[1]

It would appear therefore that radium injected intravenously or subcutaneously, either in the form of a bromide, or as an "active deposit" solution, will, after a short time, reach all parts of the animal body. Attention is called to the observation of Salant and Meyer, that radium after a subcutaneous injection is eliminated by way of the kidneys, the liver, and the small intestine. In such a case the lungs were not called upon to eliminate the radium, at least not to the extent recorded for the other organs. This observation would tend to explain the results regarding the lung changes noted in the animals of the present investigation.

In series B there was much less lung damage following a subcutaneous injection, than was recorded for the lungs in series A, where intravenous injections were given. It would appear from the work of Meyer and the present writer, that when radium is placed directly in the blood stream of an animal the lungs of that animal are exposed to a considerable amount of radioactivity.

From a careful histological study of the results of these experiments, no data have been obtained showing that the nucleoplasm of the cell is more severely damaged, or damaged more quickly, than the cytoplasm. To be sure, atypical nuclei have often been noted, but these were associated with severe degenerative changes in the cytoplasm as well. The cytoplasm in the degenerating cells was clumped in granules, and it appeared that water had been taken in by the cell to a considerable extent. This condition might very well have resulted from a change in the permeability of the cell membrane, due to the destructive action of the radium rays. It seems probable that following such a condition the cell metabolism would be interfered with, and the nucleoplastic changes that subsequently occur would be but secondary in character, or at least simultaneous but not primary.

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[1] Since the above was written the author has found that in 1914 Dr. William Duane reported a similar experiment before the American Philosophical Society. His results gave a somewhat higher proportion of deposited activity in the spleen and liver.

SUMMARY AND CONCLUSIONS

1. Following injections of an "active deposit" of radium emanation there is a diffusion of the radio-active substance throughout the animal body, which results in pathological changes in the various organs.
2. Pathological changes occurring in the liver, lungs, kidneys, adrenals, spleen, bone marrow, brain, and vascular system were presented in detail. The most interesting changes were those that were found in the liver, and resulted from comparatively small doses of radium injected subcutaneously. A fatty degeneration, associated with many giant cells and hyperchromatic nuclei, was found in the liver for a comparatively long time after the treatment.
3. Following large doses of radium, congestion and hemorrhages were frequently found in practically all the organs and in the severe, acute cases the animals died after showing symptoms of marked enteritis.
4. The most frequent pathological condition that occurred in the kidney was a granular degeneration and erosion of the renal cells.
5. It was found that injections of radium resulted in the destruction of the cells of the bone marrow, and replacement by blood.
6. Congestion of the spleen was the most constant feature following radium treatment, and in some cases this was associated with hemorrhages, and the destruction of red blood cells.
7. The method of injection appears to determine, to a certain extent, the severity of reaction in certain organs. For example, following subcutaneous injections there was comparatively no pathological reaction, of an appreciable extent, in the lungs, but with intravenous doses, of about the same strength,

the lung lesions were severe, and consisted of proliferation and desquamation of the epithelial cells of the bronchi, marked edema, congestion, and hemorrhage.

8. It appears that doses of radium less than 10 mc. are sublethal for the animals of this investigation. Doses above this amount may kill within a few hours to a few days after treatment, the reaction being somewhat less severe if the subcutaneous rather than the intravenous method of injection is used.

9. A similarity was noted in tissue reaction between radium injected intravenously or subcutaneously, and radium applied externally.

10. The fate of radium, after its injection in the animal organism and its subsequent elimination, was discussed in some detail. The results of this investigation tend to show that the liver, gastrointestinal tract, kidneys, lungs, and spleen receive the greatest amount of radio-activity.

11. Degenerative changes in the cell, and their possible interpretation were also discussed. The histological study tends to show that cytoplasmic changes occurring in the cell are profound, and as severe as the changes in the nucleoplasm.

ACKNOWLEDGMENTS

The writer wishes to acknowledge his indebtedness to Dr. James Ewing and Dr. Elise S. L'Esperance for their assistance in the interpretation of the pathological results. Thanks are due to Dr. William Duane and Mr. Gioacchino Failla for assistance in matters pertaining to the physical measurements of radium, and also to Mrs. A. Punshon, who assisted me in the preparation of the histological material connected with the experiments.

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PLATE 1

Kidney of animal VIII, series A. Death occurred two and a half days after the intravenous injection of 11.2 mc. of radium. Note the congestion and hemorrhage. (For further reference see text.)

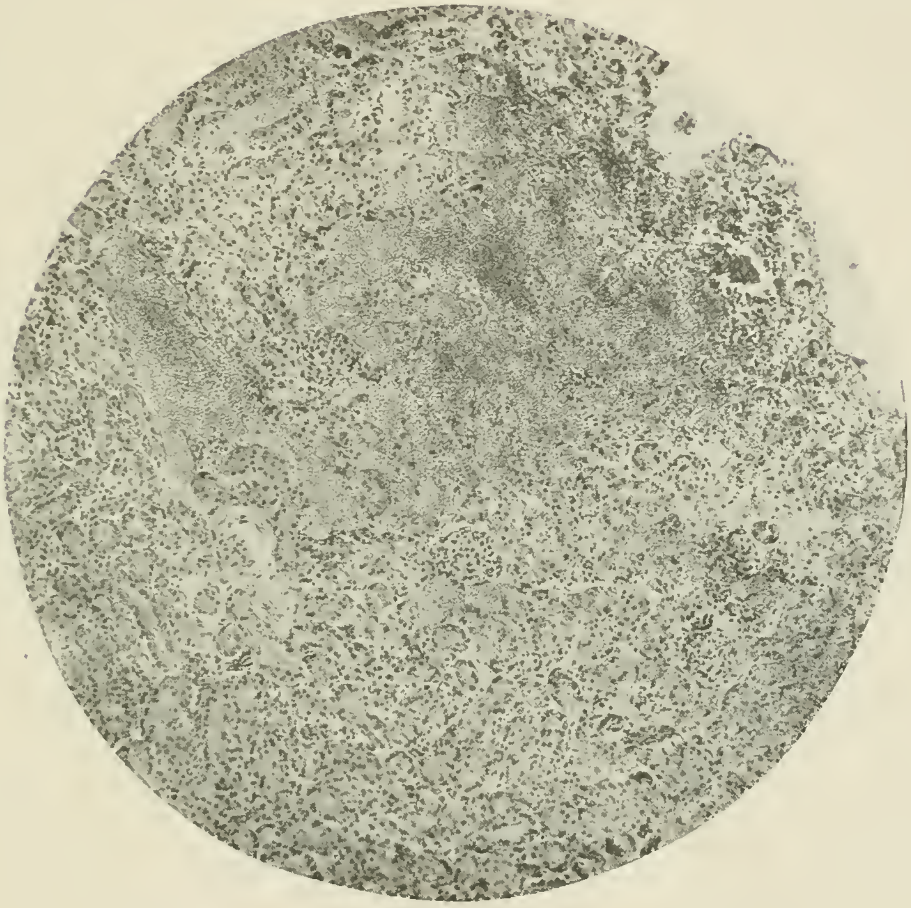
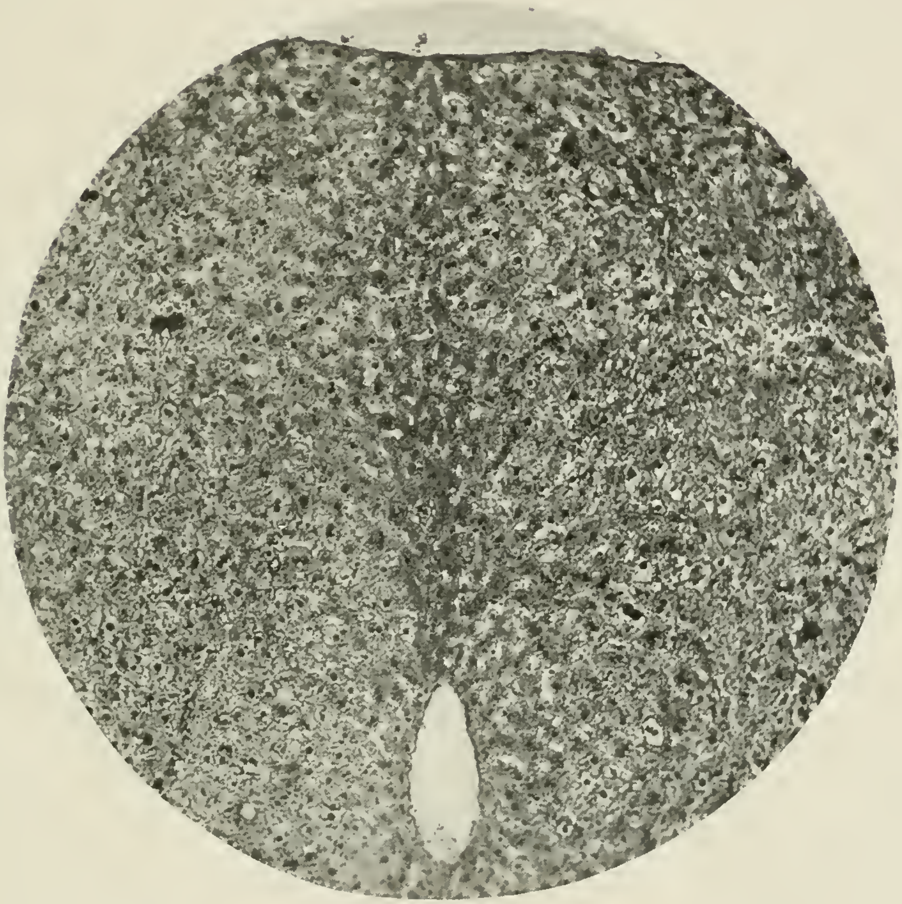


PLATE 2

Liver of animal X, series B. The animal died five days after a subcutaneous injection of 20.5 mc. of radium. The central area shows a region of fairly normal liver cells, on either side there is severe congestion and degeneration with the presence of giant cells. (For further reference see text.)



THE RELATION OF PREGNANCY AND REPRODUCTION TO TUMOR GROWTH[1]

STUDIES IN THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE

PROBLEMS IN THE BEHAVIOR OF TUMORS

Tenth Report

MAUD SLYE

From the Cancer Laboratory of the Otho S. A. Sprague Memorial Institute and the University of Chicago

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Every biological fact concerning the behavior of cancer is a step toward the solution of the problems of that disease. Biological data help to form the very foundation of the most advanced medical science today. This is particularly true in regard to neoplasms, which have in many of their aspects, including heredity, been shown to conform closely to laws which are an established part of modern biology and which are no longer under dispute.

These biological data, then, cannot be brushed aside because we do not wish to believe them[2] or ignored because they interfere with some preconceived idea.

The literature of the experimental laboratory gives little data on the influence of pregnancy upon the rate of tumor growth. Leo Loeb (2) in his paper on Tissue Growth and Tumor Growth discusses in detail the general factors modifying tumor growth. In this paper he states that "in the rat, pregnancy seems to favor growth of embryonal tissues under certain conditions; in the mouse it is unfavorable to such growth." Later in this same paper, he states "it will be necessary to distinguish more sharply than has been done in the past between the effect of pregnancy on the growth of *spontaneous* and of *transplanted* tumors." Whether the growths referred to above were spontaneous or transplanted is not stated. Woglom (3), reviewing this phase of cancer, said "It has been asserted and denied that the existence of pregnancy rendered animals less susceptible to *implantation*." Haaland found that pregnancy often exerted an inhibitory influence upon the proliferation of tumors, the effect of which was to produce a striking retardation of their growth in pregnant animals as compared with animals not bearing young.

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[1] Delivered before the American Society for Cancer Research, Atlantic City, June 14, 1919.

[2] Ewing, J.: Neoplastic Diseases, Philadelphia, 1919, p. 107: "Nothing authorizes us to affirm that cancer is hereditary. In the interests of the public this doctrine ought to be combated."

Clinical literature yields case reports from which diverse conclusions are drawn. Bainbridge (1), reporting two of his own cases, states: "from these and other personal observations, as well as from the literature, it is apparent that pregnancy exercises a stimulating and hence a malign influence on coexistent cancer in any part of the body."

Bainbridge reviews the literature, and for purposes of ready reference I have added his bibliography to this article. He states:

> Siebold maintains he has observed a spontaneous cure of genital cancer owing to a supervening pregnancy. Pinard considers the rapid growth of cancer during pregnancy by no means proved, and Varnier reports a somewhat remarkable case: "In October 1897 the presence of an enormous carcinoma of the portio was ascertained in a pregnant woman. The following year there was again a pregnancy and death did not take place until October 1900."

Ewing (5) says: "Pregnancy has an unfavorable influence on the course of many uterine carcinomas, but this influence is not always apparent."

There is, therefore, in the light of these conflicting statements, need of exact data from the experimental laboratory on this subject, which in its fundamentals is entirely a biological subject, but whose clinical importance is very great.

This paper presents exact biological data concerning the behavior of spontaneous tumors in their relation to pregnancy and reproduction. In all this work nature is allowed to take her own course and is not interfered with in any way. In order to follow the most rigid exactions, I have confined this study to tumors of a specific type and of a specific organ, viz: alveolar tubular carcinoma of the mammary gland, some portions of some of the tumors being cystic. For purposes of comparison, a few other types have been introduced.

Five years ago I reported briefly on this point (4) and the subject has been under study ever since. The data which are here given represent, therefore, a portion of the accumulated evidence of five years and are perfectly typical of the behavior of tumor growth in its relation to pregnancy and reproduction.

In handling large numbers of these mice with spontaneous tumors, there is forced upon the observer from the very first (1) the tremendous difference in the rate of tumor grown in the non-reproducing and in the reproducing females; (2) the same tremendous difference in the amounts of tumor grown by a reproductive female during her reproductive and her non-reproductive periods.

The point is of such importance in the etiology of neoplasms as to warrant a very complete analysis of the facts.

For this demonstration I have selected thirty females not reproducing after the appearance of their tumors, but all of which had previously borne young; and thirty females constantly reproducing after the appearance of their tumors, all of which also had previously borne young. The data concerning the latter are divided into two periods, (1) the reproductive and (2) the non-reproductive periods. Charts 1 to 4 concern the females not reproducing after the appearance of their tumors, and give the following information on each mouse:

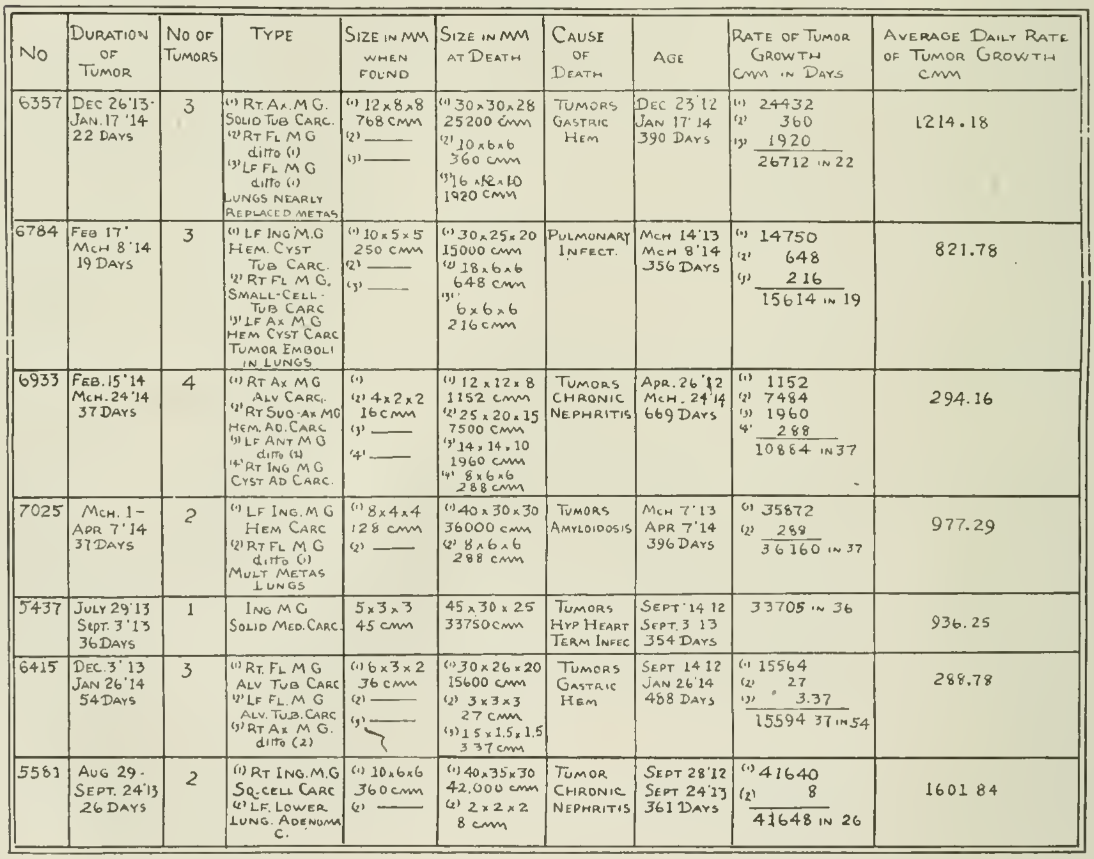
1. The duration of the tumor from the date found to the date of death.
2. The number of tumors.
3. The type of tumor.
4. Size in millimeters of the tumor when found.

5. Size of the tumor at death.
6. Causes of death.
7. Age of the mouse in days.
8. Total amount of tumor growth in cubic millimeters.
9. Average daily rate of tumor growth in cubic millimeters.

FEMALES NOT REPRODUCING AFTER THE APPEARANCE OF THEIR TUMORS

1. Female 6357 was picked up December 26, 1913, with a tumor in the right axilla 12 x 8 x 8 mm. or 768 cmm. She lived twenty-two days, during which time the first tumor became 30 x 30 x 28 mm. or 25,200 cmm. in size, while there also developed a second tumor of 360 cmm., and a third of 1920 cmm. She died January 17, 1914, of tumors and a gastric hemorrhage. She was then, at death, 1 year and 25 days, or 390 days old. During the period of tumor growth, 22 days, she grew 26,712 cmm. of tumor, an average of 1214.18 cmm. daily.

CHART 1



No	Duration of Tumor	No of Tumors	Type	Size in MM when Found	Size in MM at Death	Cause of Death	Age	Rate of Tumor Growth Cmm in Days	Average Daily Rate of Tumor Growth Cmm
6357	Dec 26'13- Jan. 17 '14 22 Days	3	(1) Rt. Ax. M G. Solid Tub Carc. (2) Rt Fl M G ditto (1) (3) Lf Fl M G ditto (1) Lungs nearly replaced metas	(1) 12 x 8 x 8 768 cmm (2) ——— (3) ———	(1) 30 x 30 x 28 25200 cmm (2) 10 x 6 x 6 360 cmm (3) 16 x 12 x 10 1920 cmm	Tumors Gastric Hem	Dec 23'12 Jan 17'14 390 Days	(1) 24432 (2) 360 (3) 1920 26712 in 22	1214.18
6784	Feb 17' Mch 8'14 19 Days	3	(1) Lf Ing M.G Hem. Cyst Tub Carc. (2) Rt Fl M G. Small-Cell-Tub Carc (3) Lf Ax M G Hem Cyst Carc Tumor emboli in lungs	(1) 10 x 5 x 5 250 cmm (2) ——— (3) ———	(1) 30 x 25 x 20 15000 cmm (2) 18 x 6 x 6 648 cmm (3) 6 x 6 x 6 216 cmm	Pulmonary Infect.	Mch 14'13 Mch 8'14 356 Days	(1) 14750 (2) 648 (3) 216 15614 in 19	821.78
6933	Feb. 15'14 Mch. 24'14 37 Days	4	(1) Rt Ax M G Alv Carc. (2) Rt Suo-Ax MG Hem. Ad. Carc (3) Lf Ant M G ditto (2) (4) Rt Ing M G Cyst Ad Carc.	(1) (2) 4 x 2 x 2 16 cmm (3) ——— (4) ———	(1) 12 x 12 x 8 1152 cmm (2) 25 x 20 x 15 7500 cmm (3) 14 x 14 x 10 1960 cmm (4) 8 x 6 x 6 288 cmm	Tumors Chronic Nephritis	Apr. 26'12 Mch. 24'14 669 Days	(1) 1152 (2) 7484 (3) 1960 (4) 288 10884 in 37	294.16
7025	Mch. 1- Apr 7'14 37 Days	2	(1) Lf Ing. M G Hem Carc (2) Rt Fl M G ditto (1) Mult Metas Lungs	(1) 8 x 4 x 4 128 cmm (2) ———	(1) 40 x 30 x 30 36000 cmm (2) 8 x 6 x 6 288 cmm	Tumors Amyloidosis	Mch 7'13 Apr 7'14 396 Days	(1) 35872 (2) 288 36160 in 37	977.29
5437	July 29'13 Sept. 3'13 36 Days	1	Ing M G Solid Med. Carc.	5 x 3 x 3 45 cmm	45 x 30 x 25 33750 cmm	Tumors Hyp Heart Term Infec	Sept '14 12 Sept. 3 13 354 Days	33705 in 36	936.25
6415	Dec. 3'13 Jan 26'14 54 Days	3	(1) Rt. Fl M G Alv Tub Carc (2) Lf Fl. M G Alv. Tub. Carc (3) Rt Ax M G. ditto (2)	(1) 6 x 3 x 2 36 cmm (2) ——— (3) ———	(1) 30 x 26 x 20 15600 cmm (2) 3 x 3 x 3 27 cmm (3) 1.5 x 1.5 x 1.5 3.37 cmm	Tumors Gastric Hem	Sept 14 12 Jan 26'14 488 Days	(1) 15564 (2) 27 (3) 3.37 15594.37 in 54	288.78
5581	Aug 29- Sept. 24'13 26 Days	2	(1) Rt Ing. M.G Sq-cell Carc (2) Lf. Lower Lung. Adenoma C.	(1) 10 x 6 x 6 360 cmm (2) ———	(1) 40 x 35 x 30 42,000 cmm (2) 2 x 2 x 2 8 cmm	Tumor Chronic Nephritis	Sept 28'12 Sept 24'13 361 Days	(1) 41640 (2) 8 41648 in 26	1601.84

2. Female 6784, was found February 17, 1914, with a tumor 10 x 5 x 5 mm., or 250 cmm., in the left inguinal mammary gland. She died at 356 days of age, the original tumor having grown 14,750 cmm., while two other tumors of 648 cmm. and 216 cmm., respectively, had developed. She grew then 15,614 cmm. of tumor in 19 days, an average of 821.78 cmm. daily, dying of pulmonary infection.

3. Female 6933 died at 669 days old, of chronic nephritis, with a tumor growth of 10,884 cmm. in 37 days, an average of 294.16 cmm.

4. Female 7025 died at 396 days, of tumors and general amyloidosis, with a total tumor growth of 36,160 cmm. in 37 days, or an average growth of 977.29 cmm.

5. Female 5437 had a tumor of 45 cmm. when found; at death, 36 days later, the tumor measured 33,705 cmm., an average growth of 936.25 cmm. daily. She died when she was 354 days old of a terminal infection, with hypertrophied heart.

6. Female 6415, sister of 5437, died of tumors and a gastric hemorrhage at 488 days of age. She grew 15,594.37 cmm. of tumor in 54 days, an average of 288.78 cmm. daily.

7. Female 5581 was picked up August 29, 1913, with a right inguinal mammary gland tumor 10 x 6 x 6 mm. (360 cmm.). She died September 24, or 26 days later, of tumor and chronic nephritis, her tumor then measuring 42,000 cmm. She had grown 41,648 cmm. of tumor in 26 days, an average daily growth of 1601.84 cmm.

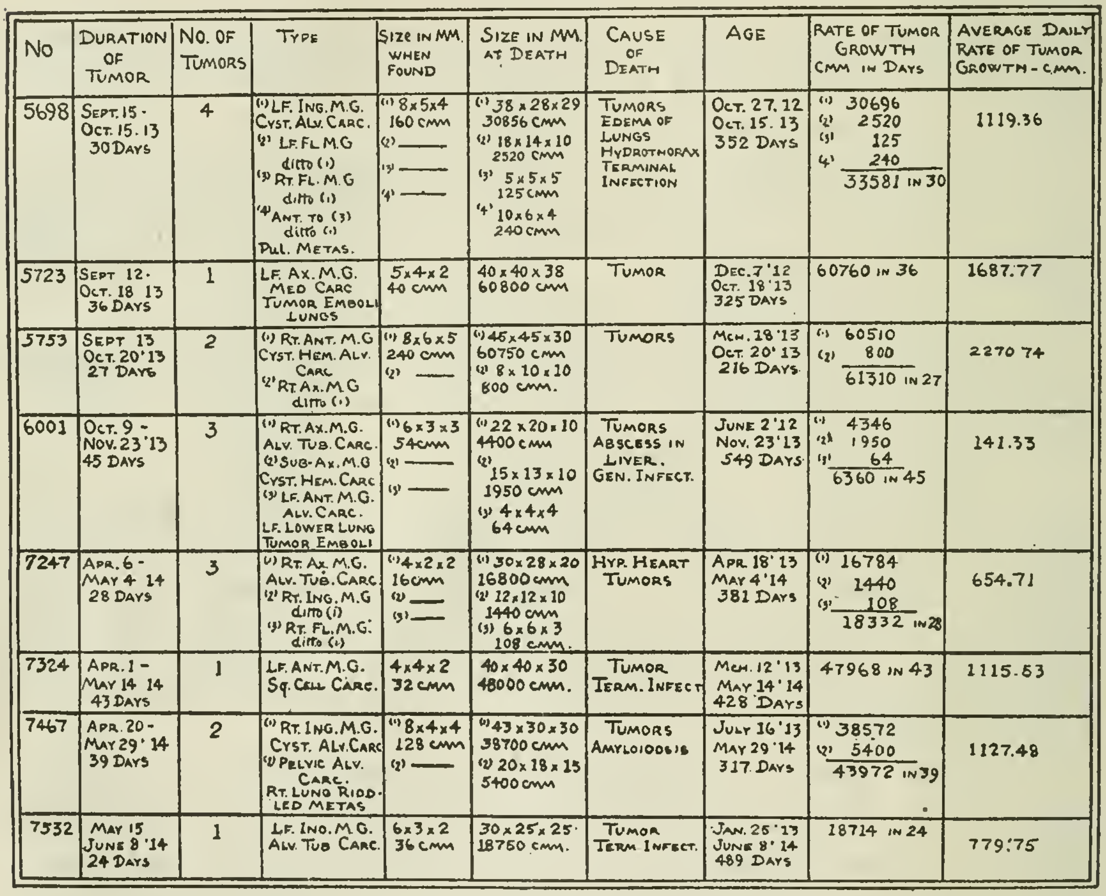
8. Female 5698 was found on September 15, 1913, with a tumor 8 x 5 x 4 mm. (160 cmm.) in the left inguinal mammary gland. She lived thirty days, during which time her first tumor grew 30,696 cmm. and she developed three other tumors of 2520, 125 and 240 cmm., respectively. She died at 352 days of age with terminal infection involving edema of the lungs and hydrothorax. She thus grew 33,581 cmm. of tumor in 30 days, an average of 1119.36 cmm.

9. Female 5723 on September 12, 1913, exhibited a left axillary mammary gland tumor 5 x 4 x 2 mm. or 40 cmm. She lived 36 days, growing 60,760 cmm. of tumor or an average of 1687.77 cmm.

At necropsy she disclosed a medullary carcinoma with multiple tumor emboli in the lungs.

10. Female 5753, sister of 5581, was found September 13, 1913, with a tumor of 240 cmm. in the right anterior mammary gland. She lived 27 days during which time her first tumor grew to be 45 x 45 x 30 mm. or 60,750 cmm., while a second tumor of 800 cmm. also developed. She grew then 61,310 cmm. of tumor in 27 days or an average of 2270.74 cmm. She died of her tumors at the age of 216 days.

CHART 2



No	Duration of Tumor	No. of Tumors	Type	Size in MM. when Found	Size in MM. at Death	Cause of Death	Age	Rate of Tumor Growth Cmm in Days	Average Daily Rate of Tumor Growth - cmm.
5698	Sept. 15 - Oct. 15. 13 30 Days	4	(1) Lf. Ing. M.G. Cyst. Alv. Carc. (2) Lf. Fl. M.G ditto (1) (3) Rt. Fl. M.G ditto (1) (4) Ant. to (3) ditto (1) Pul. Metas.	(1) 8x5x4 160 cmm (2) —— (3) —— (4) ——	(1) 38 x 28 x 29 30856 cmm (2) 18 x 14 x 10 2520 cmm (3) 5 x 5 x 5 125 cmm (4) 10 x 6 x 4 240 cmm	Tumors Edema of Lungs Hydrothorax Terminal Infection	Oct. 27. 12 Oct. 15. 13 352 Days	(1) 30696 (2) 2520 (3) 125 (4) 240 33581 in 30	1119.36
5723	Sept 12 - Oct. 18 13 36 Days	1	Lf. Ax. M.G. Med Carc Tumor Emboli Lungs	5x4x2 40 cmm	40 x 40 x 38 60800 cmm	Tumor	Dec. 7 '12 Oct. 18 '13 325 Days	60760 in 36	1687.77
5753	Sept 13 Oct. 20 '13 27 Days	2	(1) Rt. Ant. M.G Cyst. Hem. Alv. Carc (2) Rt. Ax. M.G ditto (1)	(1) 8x6x5 240 cmm (2) ——	(1) 45x45x30 60750 cmm (2) 8 x 10 x 10 800 cmm.	Tumors	Mch. 18 '13 Oct. 20 '13 216 Days	(1) 60510 (2) 800 61310 in 27	2270 74
6001	Oct. 9 - Nov. 23 '13 45 Days	3	(1) Rt. Ax. M.G. Alv. Tub. Carc. (2) Sub-Ax. M.G Cyst. Hem. Carc (3) Lf. Ant. M.G. Alv. Carc. Lf. Lower Lung Tumor Emboli	(1) 6 x 3 x 3 54 cmm (2) —— (3) ——	(1) 22 x 20 x 10 4400 cmm (2) 15 x 13 x 10 1950 cmm (3) 4 x 4 x 4 64 cmm	Tumors Abscess in Liver. Gen. Infect.	June 2 '12 Nov. 23 '13 549 Days	(1) 4346 (2) 1950 (3) 64 6360 in 45	141.33
7247	Apr. 6 - May 4 14 28 Days	3	(1) Rt. Ax. M.G. Alv. Tub. Carc. (2) Rt. Ing. M.G ditto (1) (3) Rt. Fl. M.G. ditto (1)	(1) 4x2x2 16 cmm (2) —— (3) ——	(1) 30 x 28 x 20 16800 cmm (2) 12 x 12 x 10 1440 cmm (3) 6 x 6 x 3 108 cmm.	Hyp. Heart Tumors	Apr. 18 '13 May 4 '14 381 Days	(1) 16784 (2) 1440 (3) 108 18332 in 28	654.71
7324	Apr. 1 - May 14 14 43 Days	1	Lf. Ant. M.G. Sq. Cell Carc.	4x4x2 32 cmm	40 x 40 x 30 48000 cmm.	Tumor Term. Infect	Mch. 12 '13 May 14 '14 428 Days	47968 in 43	1115.53
7467	Apr. 20 - May 29 '14 39 Days	2	(1) Rt. Ing. M.G. Cyst. Alv. Carc (2) Pelvic Alv. Carc. Rt. Lung Riddled Metas	(1) 8x4x4 128 cmm (2) ——	(1) 43 x 30 x 30 38700 cmm (2) 20 x 18 x 15 5400 cmm	Tumors Amyloidosis	July 16 '13 May 29 '14 317 Days	(1) 38572 (2) 5400 43972 in 39	1127.48
7532	May 15 June 8 '14 24 Days	1	Lf. Ing. M.G. Alv. Tub Carc.	6x3x2 36 cmm	30 x 25 x 25 18750 cmm.	Tumor Term. Infect.	Jan. 25 '13 June 8 '14 489 Days	18714 in 24	779.75

11. Female 6001 was picked up on October 9, 1913, with a tumor 6 x 3 x 3 mm. (54 cmm.) in the right axilla. She lived 45 days after this date, her first tumor growing to 4400 cmm., along with two other tumors of 1950 cmm. and 64 cmm. respectively. She died at 549 days of age, necropsy showing an abscess in the liver with generalized infection and tumor emboli in the lung, in addition to the mammary gland tumors. She grew 6360 cmm. of tumor in 45 days, an average of 141.33 cmm.

12. Female 7247 was found on April 6, 1914, with a right axillary mammary gland tumor 4 x 2 x 2 mm. (16 cmm.). She lived 28 days thereafter during which time her first tumor became 30 x 28 x 20 mm. in size (16,800 cmm.). She grew also in this period a second tumor of 1440 cmm. and a third of 108 cmm. She died at 381 days of age, having grown 18,332 cmm. of tumor in 28 days, or an average of 654.71 cmm. daily.

CHART 3.

No.	Duration of Tumor	No. of Tumors	Type	Size in mm when found	Size in mm at Death	Cause of Death	Age	Rate of Tumor Growth cmm in Days	Average Daily Rate of Tumor Growth cmm
7572	May 4 - June 16 '14 43 Days	6	(1) Lf. Fl. M. G. Tub. Carc. (2) Lf. Ax. M. G. Cyst. Tub. Carc. (3) Lf. Sub-Ax. M. G. Tub. Carc. (4) Adjacent to (3) Hem. Cyst. Carc. (5) Rt. Ant. M. O. Lymphoma (6) Lf. Ant. M. G. ditto (5)	(1) 4 x 4 x 3 48 cmm (2) 4 x 2 x 2 16 cmm (3) ——— (4) ——— (5) 4 x 4 x 4 64 cmm (6) ———	(1) 14 x 12 x 8 1344 cmm (2) 14 x 12 x 10 1680 cmm (3) 10 x 10 x 8 800 cmm (4) 5 x 5 x 5 125 cmm (5) 22 x 20 x 16 7040 cmm (6) 10 x 8 x 8 640 cmm	Tumors	Dec 5 '12 June 16 '14 558 Days	(1) 1296 (2) 1664 (3) 800 (4) 125 (5) 6976 (6) 640 11501 in 43	267.46
7761	May 25 July 8 '14 44 Days	1.	Lf. Fl. M. O. Alv. Tub. Carc. Lungs Riddled Metas	5 x 4 x 4 80 cmm	45 x 40 x 35 63000 cmm	Atrophied Organs from Tumor Pressure	July 19 '13 July 8 '14 354 Days	62920 in 44	1430.0
5357	July 15 - Aug. 25 '13 41 Days	1	Rt. Ant. M. G. Tub. Carc.	4 x 4 x 4 64 cmm	40 x 30 x 25 30000 cmm	Hyp. Heart Tumor	July 5 '12 Aug 25 '13 416 Days	29936 in 41	730.14
8042	July 5 - Aug. 13 '14 39 Days	2	(1) Rt. Ax. M. G. Alv. Tub. Carc. (2) Lf. Ing. M. G. ditto (1) Lungs Metas	(1) 5 x 3 x 3 45 cmm (2) ———	(1) 35 x 28 x 25 24500 cmm (2) 6 x 6 x 6 216 cmm	Tumors	June 30 '13 Aug. 13 '14 409 Days	(1) 24455 (2) 216 24671 in 39	632.58
8104	July 1 - Aug. 21 '14 51 Days	3	(1) Lf. Fl. M. G. Sq. cell Carc. Metas Reg. Gland (2) Rt. Ing. M. G. Alv. Tub. Carc. (3) Lf. Ing. M. G. Sq.-cell Carc. Lungs Replaced Metas	(1) 6 x 5 x 3 90 cmm (2) ——— (3) ———	(1) 45 x 40 x 35 63000 cmm (2) 2 x 2 x 2 8 cmm (3) 1.5 x 2 x 2 6 cmm	Tumors Terminal Infect	Oct 2 '13 Aug 21 '14 323 Days	(1) 62910 (2) 8 (3) 6 62924 in 51	1233.80
8573	Sept 15 - Oct 23 '14 38 Days	3	(1) Rt. Fl. M. G. Alv. Tub. Carc. (2) Lf. Ant. M. G. ditto (1) (3) Lf. Ax. M. G. ditto (1)	(1) 5 x 4 x 2 40 cmm (2) ——— (3) ———	(1) 40 x 30 x 30 36000 cmm (2) 3 x 3 x 3 27 cmm (3) 2 x 2 x 2 8 cmm	Tumors	Oct. 28 '13 Oct. 23 '14 359 Days	(1) 35960 (2) 27 (3) 8 35995 in 38	947.23
8911	Oct. 29 - Dec 6 '14 38 Days	1	Rt. Ax. M. G. Alv. Ad. Carc. Lungs Mult. Emboli	8 x 5 x 3 120 cmm	45 x 40 x 35 63000 cmm	Tumors	Apr. 23 '14 Dec 6 '14 227 Days	62880 in 38	1654.73

A study of the charts will show parallel data concerning each of the other eighteen individuals in the class of mice which did not reproduce after the appearance of a tumor; in order to conserve space the other case reports are given in the charts only. The average daily rate of growth for the total number of these mice was 999.45 cmm. The youngest of these mice was 216 days, the oldest 803 days. The average age then, was 415 days or 1 year, 1 month, 21 days.

The second set of charts, nos. 5 to 8, deals with the reproducing females and gives the same items as the first set with the addition of the following:

10. The number of litters born after the appearance of tumor.
11. The total number of young after the appearance of tumor.
12. Time between the date of the last litter and the death date.

The total tumor growth and the average daily rate of growth are given in two groups: (1) the reproductive period, (2) the non-reproductive period.

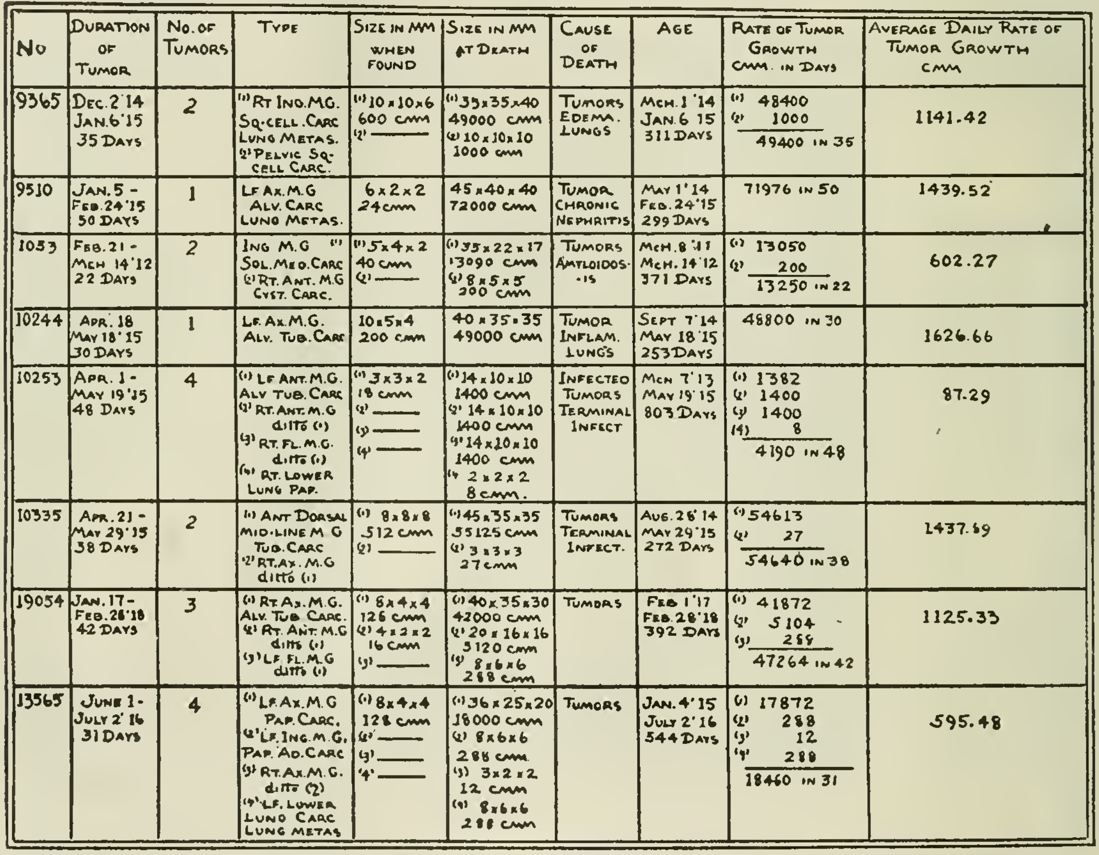


CHART 4

No	Duration of Tumor	No. of Tumors	Type	Size in mm when found	Size in mm at Death	Cause of Death	Age	Rate of Tumor Growth cmm. in Days	Average Daily Rate of Tumor Growth cmm
9365	Dec. 2 '14 Jan. 6 '15 35 Days	2	(1) Rt Ing. M.G. Sq-cell. Carc Lung Metas. (2) Pelvic Sq-cell Carc.	(1) 10 x 10 x 6 600 cmm (2) ———	(1) 35 x 35 x 40 49000 cmm (2) 10 x 10 x 10 1000 cmm	Tumors Edema. Lungs	Mch. 1 '14 Jan. 6 '15 311 Days	(1) 48400 (2) 1000 49400 in 35	1141.42
9510	Jan. 5 - Feb. 24 '15 50 Days	1	Lf Ax. M.G Alv. Carc Lung Metas.	6 x 2 x 2 24 cmm	45 x 40 x 40 72000 cmm	Tumor Chronic Nephritis	May 1 '14 Feb. 24 '15 299 Days	71976 in 50	1439.52
1053	Feb. 21 - Mch 14 '12 22 Days	2	Ing M.G (1) Sol. Med. Carc (2) Rt. Ant. M.G Cyst. Carc.	(1) 5 x 4 x 2 40 cmm (2) ———	(1) 35 x 22 x 17 13090 cmm (2) 8 x 5 x 5 200 cmm	Tumors Amyloidosis	Mch. 8 '11 Mch. 14 '12 371 Days	(1) 13050 (2) 200 13250 in 22	602.27
10244	Apr. 18 May 18 '15 30 Days	1	Lf. Ax. M.G. Alv. Tub. Carc	10 x 5 x 4 200 cmm	40 x 35 x 35 49000 cmm	Tumor Inflam. Lungs	Sept 7 '14 May 18 '15 253 Days	48800 in 30	1626.66
10253	Apr. 1 - May 19 '15 48 Days	4	(1) Lf Ant. M.G. Alv Tub. Carc (2) Rt. Ant. M.G ditto (1) (3) Rt. Fl. M.G. ditto (1) (4) Rt. Lower Lung Pap.	(1) 3 x 3 x 2 18 cmm (2) ——— (3) ——— (4) ———	(1) 14 x 10 x 10 1400 cmm (2) 14 x 10 x 10 1400 cmm (3) 14 x 10 x 10 1400 cmm (4) 2 x 2 x 2 8 cmm.	Infected Tumors Terminal Infect	Mch 7 '13 May 19 '15 803 Days	(1) 1382 (2) 1400 (3) 1400 (4) 8 4190 in 48	87.29
10335	Apr. 21 - May 29 '15 38 Days	2	(1) Ant Dorsal Mid-line M.G Tub. Carc (2) Rt. Ax. M.G ditto (1)	(1) 8 x 8 x 8 512 cmm (2) ———	(1) 45 x 35 x 35 55125 cmm (2) 3 x 3 x 3 27 cmm	Tumors Terminal Infect.	Aug. 28 '14 May 29 '15 272 Days	(1) 54613 (2) 27 54640 in 38	1437.89
19054	Jan. 17 - Feb. 28 '18 42 Days	3	(1) Rt. Ax. M.G. Alv. Tub. Carc. (2) Rt. Ant. M.G ditto (1) (3) Lf. Fl. M.G ditto (1)	(1) 8 x 4 x 4 128 cmm (2) 4 x 2 x 2 16 cmm (3) ———	(1) 40 x 35 x 30 42000 cmm (2) 20 x 16 x 16 5120 cmm (3) 8 x 6 x 6 288 cmm	Tumors	Feb 1 '17 Feb. 28 '18 392 Days	(1) 41872 (2) 5104 (3) 288 47264 in 42	1125.33
13565	June 1 - July 2 '16 31 Days	4	(1) Lf. Ax. M.G Pap. Carc. (2) Lf. Ing. M.G. Pap. Ad. Carc (3) Rt. Ax. M.G. ditto (2) (4) Lf. Lower Lung Carc Lung Metas	(1) 8 x 4 x 4 128 cmm (2) ——— (3) ——— (4) ———	(1) 36 x 25 x 20 18000 cmm (2) 8 x 6 x 6 288 cmm (3) 3 x 2 x 2 12 cmm (4) 8 x 6 x 6 288 cmm	Tumors	Jan. 4 '15 July 2 '16 544 Days	(1) 17872 (2) 288 (3) 12 (4) 288 18460 in 31	595.48

FEMALES CONSTANTLY REPRODUCING AFTER THE APPEARANCE OF TUMORS

1. Female 2426 was found June 16, 1912, with a tumor 10 x 5 x 5 mm. (250 cmm.) in the left inguinal mammary gland. She lived until September 7, 1912, or 83 days after the appearance of her tumor, during which time she produced 4 litters, with a total of 19 young. The last litter was born dead the day

CHARTS.

No.	Duration of Tumor	No. of Litters	No. of Young	No. of Tumors	Type.	Size in MM. When Found	Size in MM., At Death.	Cause of Death.	Time — Last Litter To Death	Age	Rate of Tumor Growth CMM in Days	Average Daily Rate of Tumor Growth CMM.
2426	June 16 — Sept. 7 '12 83 Days	4	19	1	Lf. Ing Tub. Carc. M.G.	10x5x5 250 Cmm.	15x15x15 3375 Cmm.	Hyp. Heart Thromb. Aur. Hydrothorax.	Sept. 6. (dead) Sept. 7 '12	June 27 '11 Sept. 7 '12 437 Days	3125 in 83	37.65 Reprod
3621	July 21 '12 Jan. 16 '13 179 Days	7	25	1	Rt. Ax. Alv. Tub Carc. M G	3x3x2 18 cmm Dec. 25 '12 8x3x2 48 cmm	16x8x8 1024 Cmm	Inf. with Liver Necrosis.	Dec. 25. '12 (dead) Jan. 16 '13	Dec. 5 '11 Jan. 16 '13 407 Days	30 in 157 976 in 22	.19 Reprod. 44.36 Non-Reprod.
4339	Sept. 16 '12 April 13 '13 210 Days	7	20	3	(1) Fl. to Fl Sm cell Carc. M.G. (2) Rt. ant. Carc. M.G. (med-metas) (3) Lf. Lower Lung Pad Mult. Lung Metas	4x3x2 24 cmm Feb. 21 10x8x8 640 cmm	(1) 45x45x30 60750 cmm. (2) 2x2x2 8 cmm. (3) 4x4x4 64 cmm.	Tumors	Feb. 21 (dead) April 13 '13	Sept. 10 '11 April 13 '13 580 Days.	(1) 616 in 159 60110 in 51 (2) 8 in 51 (3) 64 in 51 60182 in 51	3.87 Reprod. 1182. Non-Reprod.
4554	Jan. 30 — May 15 '13 105 Days	4	15	1	Rt. Ax Tub Carc M G	6x4x2 48 cmm April 30 6x6x6 216 cmm.	10x10x10 1000 cmm	Gen. Sepsis from Infected Uterus with Dead Foetus	April 30 (dead) - May 15 '13	Aug. 6 '12 May 15 '13 282 Days	168 in 90 784 in 15	1.86 Reprod 52.26 Non-Reprod
4751	Feb 13 — June 9 '13 116 Days	4	25	1	Rt Ax Alv Tub Carc M.G Mult. Small Metas. Lungs	10x4x3 120 cmm May 9 '13 10x8x8 640 cmm.	42x32x18 24192 cmm	Tumor	May 9. — June 9. '13	July 3 '12 June 9 '13 341 Days	520 in 85 23552 in 31	6.11 Reprod 759.74 Non-Reprod
5417	Oct 24 '12 Sept. 1 '13 311 Days	7	23	3	(1) Ing. M.G. Lar. Cell Alv. Carc (2) Pelvic Lar. Cell Carc (3) Rt. Fl. M.G Tub. Cyst. Carc. Mult. Lung Metas from (3)	(1) 8x5x5 200 cmm July 1 '13 10x8x6 480 cmm	(1) 52x42x28 61152 cmm. (2) 14x14x14 2744 cmm (3) 4x4x4 64 cmm	Tumors	July 1 (dead) Sept. 1. '13	April 30 '12 Sept. 1 '13 488 Days	(1) 280 in 250 60672 in 61 (2) 2744 in 61 (3) 64 in 61 63480 in 61	(1) 1.12 Reprod 994.62 Non- " (2) 44.98 " " (3) 1.04 " " 1040.64 Non-Rep.
5673	Dec. 31 '12 - Oct. 11 '13 284 Days	6	24	2	(1) Rt Ax. M.G. Tub. Cyst. Carc (2) Lf Ing. M.G Tub Carc	(1) 8x5x5 200 cmm June 5 10x8x8 640 cmm	(1) 35x30x18 18900 cmm. (2) 10x10x10 1000 cmm	Chr Neph	June 5 - Oct. 11 '13	Dec. 10 '10 - Oct. 11 '13 1024 Days	(1) 440 in 156 17360 in 128 (2) 1000 in 128 18360 in 128	(1) 2.82 Reprod 135.62 Non-Rep. (2) 7.81 " " 143 43 Non. Rep
6981	Oct. 1 '13 Mch. 31 '14 181 Days	6	30	1	Lf. Fl. M.G. Alv Carc	6x5x4 120 cmm Feb. 20 '14 10x8x8 640 cmm	25x15x15 5625 cmm	Infected Tumor. Gen. Sepsis - Amyloidosis	Feb. 20 '14 (dead) Mch. 31 '14	Jan. 30 '13 March 31 '14 425 Days	520 in 142 4985 in 39	2.95 Reprod 127.80 Non-Rep.
7246	Dec. 19 '13 May 5 '14 137 Days	5	21	1	Rt. to Lf Ax. M G Alv Tub Carc Mult Lung Metas Leukemia	8x5x5 200 cmm. Apr. 4 '14 8x8x8 512 cmm	34x34x30 34680 cmm	Tumor and Leukemia	Apr. 4 — May 5 '14	June 2 '13 May 5 '14 337 Days	312 in 106 34168 in 31	2.94 Reprod 1102.19 Non-Rep.

before her death, so that she had no non-reproductive period after the appearance of her tumor. She died at 437 days of age, of a hypertrophied heart with thrombosis of the left auricle and hydrothorax. She grew 3125 cmm. of tumor in 83 days, an average of 37.65 cmm. daily during her reproductive period.

2. Female 3621 was found July 21, 1912 with a right axillary mammary gland tumor 3 x 3 x 2 mm. (18 cmm.). She lived 179 days after the appearance of her tumor. She produced 25 young in 7 litters during this period. The date of her last litter, born dead, was December 25, 1912, after which she lived 22 days. She had then 179 days of tumor growth during 157 of which she was reproducing; during the remaining 22 days she had no young. During her reproductive period she grew 30 cmm. of tumor in 157 days, an average daily growth of 0.19 cmm. During her non-reproductive period of 22 days she grew 976 cmm. of tumor, an average daily growth of 44.36 cmm.

3. Female 4339 was found September 15, 1912, with a tumor 4 x 3 x 2 mm. (24 cmm.) in the right flank mammary gland. She produced 20 young in 7 litters while growing her tumor. The last litter was born dead February 21, 1913. She lived 210 days after the appearance of her tumor during which time her first tumor grew to 45 x 45 x 30 mm. (60,750 cmm.) and a second and third tumor of 8 cmm. and 64 cmm. respectively developed. She died at the age of 580 days, having grown 616 cmm. of tumor in the 159 days she was reproductive, or an average of 3.87 cmm. daily. During her non-reproductive period of 51 days, she grew 60,182 cmm. of tumor, or a daily average of 1182 cmm.

4. Female 4554 was found January 30, 1913, with a tumor 6 x 4 x 2 mm. (48 cmm.), after which she produced 4 litters with a total of 15 young. Her last litter was delivered dead, on April 30, at which time her tumor measured 6 x 6 x 6 mm. (216 cmm.). She died May 15, at 282 days of age, of general sepsis from infected uterus with a dead undelivered fetus. At her death her tumor measured 10 x 10 x 10 mm. or 1000 cmm. She grew then during her reproductive period 1.86 cmm. daily; while during her non-reproductive period she grew 52.26 cmm. daily.

5. Female 4751 on February 13, 1913, showed a right axillary mammary gland tumor 10 x 4 x 3 mm. (120 cmm.). Between that date and May 9, she had 4 litters, a total of 25 young. On the date of her last litter her tumor measured 10 x 8 x 8 mm. (640 cmm.). Between May 9 and June 9, her tumor grew to 42 x 32 x 18 mm. (24,192 cmm.). She showed at necropsy multiple lung metastases. During her reproductive period, 85 days, she grew 520 cmm., an average of 6.11 cmm. daily. During her non-reproductive period, 31 days, she grew 23,552 cmm. of tumor, 759.74 cmm. average daily growth.

6. Female 5417 was found October 24, 1912, with an inguinal mammary gland tumor 8 x 5 x 5 mm. (200 cmm.). She bore 7 litters after this date, a total of 23 young, the last a litter of 1 born dead July 1, 1913. On this latter date her tumor was still only 10 x 8 x 6 mm. (480 cmm.). She lived until September 1, 1913, her tumor at death being 52 x 42 x 28 mm. (61,152 cmm.). She had also grown a pelvic tumor 14 x 14 x 14 mm. (2744 cmm.) and a right flank mammary gland tumor of 64 cmm. At necropsy her lungs showed multiple small metastases. During her reproductive period then, 250 days, she grew only 280 cmm. of tumor, an average of 1.12 cmm. daily. During her non-reproductive period of 61 days she grew 63,480 cmm., an average of 1040.64 cmm. daily.

7. Female 5673 was picked up December 31, 1912, with a right axillary mammary gland tumor 8 x 5 x 5 mm. (200 cmm.). She bore 6 litters, a total of 24 young, after this date. Her last litter was born June 5, 1913, at which date her tumor measured 10 x 8 x 8 mm. (640 cmm.). She died October 11, 1913, of chronic nephritis, being 1024 days old (2 years, 9 months, 24 days). She showed at necropsy a second tumor 10 x 10 x 10 mm. (1000 cmm.). During her reproductive period, 156 days, she grew 440 cmm. of tumor or an average of 2.82 cmm. daily, while during her non-reproductive period, 128 days, she grew 18,360 cmm. or 143.43 cmm. daily.

8. Female 7246 was found December 19, 1913, with a right axillary mammary gland tumor 8 x 5 x 5 mm. (200 cmm.). She bore 5 litters totalling 21 young after this date. Her last

CHART 6

No	Duration of Tumor	No. of Litters	No. of Young	No. of Tumors	Type	Size in mm when Found.	Size in mm at Death	Cause of Death	Time-Last Litter to Death	Age	Rate of Tumor Growth Cmm in Days	Average Daily Rate of Tumor Growth ·Cmm
7454	Sept 26 13 May 26 14 242 Days	6	20	1	Rt to Lf Ax. M.G. Sp Cell Sarcoma	10x10x10 1000 cmm. May 1'14 12x12x12 1728 cmm	42x40x30 50400 cmm	Tumor	May 1- May 26 14	Feb. 21'13 May 26'14 459 Days	728 in 217 48672 in 25	3.35 Reprod 1946.88 Non-Rep
7536	Feb 20 June 9 14 109 Days	5	19	1	Lf Ax M.G Par Ad. Carc Lungs Riddled Metas	6x6x6 216 cmm May 22'14 8x6x6 288 cmm.	14x12x10 1680 cmm	Nematodes Tapeworm Typ. Changes	May 22 June 9' 14	June 23'13 June 9 '14 351 Days	72 in 91 1392 in 18	.79 Reprod 77.33 Non-Rep
7555	Jan. 13'14 June 13'14 151 Days	4	18	3	(1) Rt. Ant M.G. Alv Tub Carc (2) Rt Ax M.G Scirr. Carc (3) Lf. Ax. M.G Scirr Carc Mult Lung Metas.	(1) 8x8x8 512 cmm May 7'14 10x8x10 800 cmm	(1) 18x18x14 4536 cmm (2) 10x10x10 1000 cmm (3) 5x5x5 125 cmm	Hyp. Heart. Lung Tumors	May 7 – June 13'14	Jan. 19 13 June 13'14 510 Days	(1) 288 in 114 3736 in 37 (2) 1000 in " (3) 125 " " 4861 in 37	(1) 2.52 Reprod 100.97 Non-Rep. (2) 27 02 " (3) 3.37 " 131 36 Non Rep
8304	Mch. 14 Sept 15'14 185 Days	4	17	1	Rt Ant M G Cyl cell Carc	4x4x4 64 cmm June 1'14 5x5x5 125 cmm	10x10x10 1000 cmm	Hyp. Heart Hydro- thorax.	June 1- Sept. 15'14	Sept. 5'12 Sept 15'14 740 Days	61 in 79 875 " 106	.77 Reprod 8.25 Non-Rep
8889	July 19 Dec 3'14 137 Days	5	20	4	(1) Lf to Rt Ax. M.G 3 Nods Alv. Tub. pap Carc Sp. cell Sarc Tub Carc (2) Lf. Fl. M.G Early Cyst. Carc Lungs Riddled Metas	10x10x10 1000 cmm Nov. 19'14 20x20x20 8000 cmm	(1) 50x30x30 45000 cmm (2) 2x2x2 8 cmm	Tumors	Nov. 19.– Dec 3'14	Dec 7'13 Dec 3'14 392 Days	(1) 7000 in 123 37000 " 14 (2) 8 " " 37008 " 14	(1) 56.91 Reprod 2462 85 Non " (2) 57 " " 2643.42 Non-R.
9053	Sept 2 - Dec. 27'14 116 Days	4	18	1	Lf Ax M.G. Sol Alv Carc.	6x6x6 216 cmm Dec 21'14 7x6x6 252 cmm	10x10x8 800 cmm	Tape- worm Chr. Neph.	Dec 21.- Dec. 27'14	Mch. 9'14 Dec 27'14 293 Days	36. in 110 548. in 6	32 Reprod 91.33 Non-R.
9097	Nov. 5'14 Jan. 2 15 58 Days	3	10	1	Rt Ing. M.G. Alv Tub Carc	10x10x10 1000 cmm Dec 20'14 10x10x10 1000 cmm	14x12x10 1680 cmm	Hem Left Ovary	Dec. 20'14 Jan 2'15	Mch. 12'13 Jan 2'15 661 Days	0. in 45 680 in 13	0. Reprod 52 30 Non-R

litter was born April 4, 1914, at which date her tumor measured only 8 x 8 x 8 mm. (512 cmm.). She died May 5, 1914, of tumors and leukemia, when she was 337 days old. She grew during her reproductive period, 106 days, 2.94 cmm. of tumor daily. During her non-reproductive period, 31 days, she grew 34,168 cmm. or 1102.19 cmm. daily.

9. The most striking illustration in this set of females is number 9172. She was picked up August 30, 1914, with a tumor 10 x 10 x 10 mm. (1000 cmm.) in the right axilla. After this date she bore 4 litters totalling 15 young. The date of her last litter was December 1, 1914, when her tumor measured only 11 x 11 x 10 mm. (1210 cmm.), having grown but 210 cmm. in 93 days or 2.25 cmm. daily. She lived 40 days longer and died January 10, 1915, of tumors, aged 337 days. During her non-reproductive period her tumor grew 98,790 cmm., while a left axillary carcinoma also developed to the size of 12 x 6 x 6 mm. (432 cmm.) making a total tumor growth of 99,222 cmm. in 40 days or 2480.55 cmm. daily (Chart 8).

10. Female 9097 is an interesting case. She was an old mouse, 661 days old when she died. She was found November 5, 1914, with a right inguinal mammary gland tumor 10 x 10 x 10 mm. (1000 cmm.). She bore 3 litters, 10 young, after this date. Her last litter was born December 20, 1914, at which date her tumor measured the same as when found, 45 days earlier. She lived 13 days longer, dying January 2, 1915, her tumor then being 14 x 12 x 10 mm. (1680 cmm.). She grew then 680 cmm. of tumor in 13 days while she was non-reproductive, an average of 52.3 cmm. daily. Necropsy showed a blood clot in the left ovary (Chart 8).

Charts 7 and 8 which cover the rest of the thirty reproducing females, show the same marked increase in tumor growth during the non-reproductive period. The average daily tumor growth for this set of mice was as follows:

1. During the reproductive period 7.75 cmm. daily.
2. During the non-reproductive period 686.34 cmm. daily, or over 88 times as much daily tumor growth as during the reproductive period.

CHART 7.

No.	Duration of Tumor	No. of Litters	No. of Young	No. of Tumors	Type	Size in mm when found	Size in mm at Death	Cause of Death	Time-Last Litter to Death.	Age	Rate of Tumor Growth Cmm. in Days	Average Daily Rate of Tumor Growth—
10015	Nov. 21 '14 Apr. 3 '15 133 Days	5	15	2	(1) Rt. Ing. M.G. Alv. Sarcoma Metas Lymph Gland (2) Rt. Ax. M.G. Partly Cyst. Sq. Partly Pap. Ad. Carc.	(1) 10 x 10 x 10 1000 cmm Mch. 21 '15 12 x 10 x 10 1200 cmm (2) ———	(1) 20 x 20 x 18 7200 cmm (2) 10 x 10 x 2 200 cmm	Liver Abscess Amyloidosis	Mch. 21 – Apr. 3 '15	Mch. 11 '14. Apr. 3 '15 388 Days	(1) 200 in 120 6000 in 13 (2) 200 " " 6200 in 13	(1) 1.66 Reprod 461.53 Non-Rep (2) 15.38 " " 476.91 Non-Rep.
12189	Apr. 1 '15 Jan. 21 '16 295 Days	6	26	2	(1) Rt. Fl. M.G. Alv. Tub. Cyst. Carc. (2) Lf. Fl. M.G. Ditto (1)	(1) 8 x 8 x 8 512 cmm Dec 10 '15 10 x 10 x 10 1000 cmm (2) ———	(1) 25 x 20 x 20 10000 cmm (2) 4 x 4 x 4 64 cmm	Chr. Neph	Dec. 10 '15 Jan. 21 '16	Apr. 26 '14 Jan. 21 '16 655 Days	(1) 488 in 253 9000 " 42 (2) 64 " " 9064 in 42	(1) 1.92 Reprod 214.28 Non-R. (2) 1.52 " " 215.80 Non-Rep
12852	Aug. 18 '15 Apr 18 16 244 Days	8	31	3	(1) Lf. Ax. M.G. Sol. Alv. Carc (2) Lf. Fl. M.G Cyst. Hem. Carc (3) Rt. Mid. M.G Ditto (2)	(1) 8 x 8 x 8 512 cmm. Mch. 1 '16 12 x 10 x 8 960 cmm (2) ——— (3) ———	(1) 30 x 25 x 25 18750 cmm (2) 20 x 18 x 18 6480 cmm (3) 10 x 6 x 6 360 cmm	Tumors	Mch. 1 '16 (dead) Apr. 18 '16	Jan. 30 '15 Apr. 18 '16 443 Days	(1) 448 in 196 17790. " 48 (2) 6480 " " (3) 360 " " 24630 in 48	(1) 2.28 Reprod. 370.62 Non-Rep. (2) 135. " " (3) 7.5 " " 513.12 Non-Rep.
12963	Aug 3 15 May 3 16 274 Days	7	27	2	(1) Lf. Ant. M.G. to Dor. Mid-line Alv Carc (2) Rt Mid-Lung Pap Carc	(1) 8 x 8 x 8 512 cmm Apr 1 '16 12 x 10 x 10 1200 cmm (2) ———	(1) 20 x 18 x 18 6480 cmm (2) 8 x 6 x 6 288 cmm	Hem. Ovary Lung Tumor	Apr 1 May 3 '16	Jan. 4 15 May 3 '16 485 Days	(1) 678 in 242 5280 " 32 (2) 288 " " 5568 " "	(1) 2.80 Reprod 161 87 Non-R (2) 9. " " 170 87 Non-R.
15650	Mch 15 16 " 4 17 354 days	6	22	2	(1) Rt. Ing. M.G. Hem. Alv. Tub. Carc (2) Rt. Ant. M.G Ditto (1)	(1) 4 x 4 x 4 64 cmm Feb. 5 '17 10 x 8 x 5 400 cmm (2) ———	(1) 20 x 20 x 20 8000 cmm (2) 8 x 8 x 8 512 cmm	Intest. Infect.	Feb. 5 – Mch 4 17	Aug 12 '15 Mch. 4 '17 569 Days	(1) 334 in 327 7600 " 27 (2) 512 " " 8112 in 27	(1) 1.02 Reprod 281 48 Non-Rep. (2) 18.96 299.44 Non-Rep.
16046	Nov 16 16 Apr 18 17 153 Days	5	17	2	(1) Rt. Ing. M.G Tub. Alv Carc (2) Rt Sub-ax. M.G (Ditto (1))	(1) 4 x 4 x 4 64 cmm Mch 11 '17 8 x 8 x 8 512 cmm (2) Mch. 27 '17 10 x 10 x 10 1000 cmm	(1) 18 x 16 x 16 4608 cmm (2) 11 x 11 x 11 1331 cmm	Chr. Neph.	Mch. 27. Apr 18 '17	Nov. 25 '15 Apr. 18 '17 509 Days	(1) 448 in 131 (2) 1000 in 131 1448 in 131 (1) 4096 in 22 (2) 331 " " 4427 in 22	(1) 3.41 Reprod (2) 7.63 " 11.04 Reprod (1) 186.18 Non-Rep (2) 15.04 " " 201.22 Non-Rep.
15990	Dec. 13 '16 Apr. 12 '17 120 Days	3	13	1	Lf. Ax. M.G Hem Cyst. Carc.	8 x 8 x 8 512 cmm Feb 28 '12 12 x 12 x 12 1728 cmm	40 x 40 x 40 64000 cmm	Tumor Pul Inf with Hem.	Feb. 28 (dead) Apr. 12 '17	Apr 30 '16 Apr 12 '17 348 Days	1216 in 77 62272 in 43	15.79 Reprod 1448.18 Non-Rep

CHART 8

No.	Duration of Tumor	No. of Litters	No. of Young	No. of Tumors	Type	Size in MM when Found.	Size in MM at Death	Cause of Death	Time - Last Litter to Death	Age	Rate of Tumor Growth - CMM in Days	Average Daily Rate of Tumor Growth CMM
16371	Dec. 18 '16 May 22 '17 155 days	3	7	2	(1) Rt. Ax. M.G Alv Tub. Carc (2) Lft. Ax. M.G. Ditto (1)	(1) 8 x 8 x 8 512 Cmm. Feb. 28 10 x 10 x 10 1000 Cmm (2) ———	(1) 35 x 35 x 30 36750 Cmm (2) 10 x 10 x 10 1000 Cmm	Hydrothorax Inf. Areas Lung	Feb. 28 '17 May 22 '17	May 1 '16 May 22 '17 387 Days	(1) 488 in 72 35750 in 83 (2) 1000 in 83 36750 in 83	(1) 6.77 Reprod 430.72 Non-Rep (2) 12.04 - - 442.76 Non-Rep.
18845	Feb. 19 '17 Feb. 1 '18 347 days	6	32	2	(1) Rt. Ing. M.G. Alv Carc (2) Rt. Ant. M.O. to Spine Ditto (1) Every Lobe Lung nearly Replaced - Metas	(1) 4 x 4 x 4 64 cmm Dec. 9 '17 8 x 8 x 8 512 cmm (2) Nov 25 '17 6 x 6 x 6 216 cmm	(1) 25 x 20 x 18 9000 Cmm (2) 25 x 25 x 25 15625 Cmm	Tumors Acute Neph	Nov. 25 '17 Feb. 1 '18	July 5 '16 Feb 1 '18 576 Days	(1) 448 in 279 (2) 216 in 279 664 in 279 (1) 8488 in 68 15409 in 68 23897 in 68	(1) 1.60 Reprod (2) .77 " 2.37 Reprod (1) 124.82 Non-Rep. (2) 226.60 " " 351.42 Non-Rep.
17466	May 16 '17 Aug. 30 '17 106 days	5	13	1	Rt. Ax. M.G Alv Tub. Carc.	4 x 4 x 4 64 cmm. Aug 14 '17 8 x 8 x 8 512 Cmm	18 x 18 x 18 5832 cmm	Chr Neph.	Aug. 14 - Aug 30 '17	Oct. 1 '16 Aug. 30 '17 333 Days	448 in 90 5320 in 16	4.97 Reprod. 332.50 Non-Rep.
15768	(1) Oct. 23 '16 Mch. 19 '17 (2) Feb. 27 '17 Mch. 19 '17 (3) T. Eye Sept. 29 '16 Mch. 19 '17 147 days	4	18	3	(1) Lft. Ing. M.G. Cyst. Alv. Tub. - Carc. (2) Lft Sub. Ax. M.G. Alv. Tub. Carc. (3) Lft. Lower Lung nearly Replaced - Pap.	(1) 8 x 8 x 8 512 Cmm Feb. 27 '17 12 x 12 x 12 1728 Cmm (2) 10 x 10 x 10 1000 Cmm (3) ———	(1) 25 x 25 x 25 15625 cmm (2) 25 x 20 x 20 10000 cmm (3) 10 x 10 x 10 1000 cmm	Tumors Chr Neph	Feb. 27 (dead) March 19 '17	Apr. 1 '16 Mch 19 '17 352 Days	(1) 1216 in 127 13897 in 20 (2) 9000 in 20 (3) 1000 in 20 23897 in 20	(1) 9.57 Reprod 694.85 Non-Rep. (2) 450 00 " " (3) 50.00 " " 1194. 85 Non-Rep.
9172	Aug. 30 '14 Jan 10 '15 133 days	4	15	2	(1) Rt Ax. M.G Alv. Carc. (2) Lf. Ax M.G Pap. Carc Lungs Riddled Metas	(1) 10 x 10 x 10 1000 cmm Dec. 1 '14 11 x 11 x 10 1210 cmm (2) ———	(1) 50 x 50 x 40 100000 cmm (2) 12 x 6 x 6 432 cmm	Tumors	Dec. 1 '14 Jan 10 '15	Feb 7 '14 Jan. 10 '15 337 days	(1) 210 in 93 98790 in 40 (2) 432 in - 99222 in 40	(1) 2.25 Reprod 2469.75 Non-Reprod (2) 10.8 2480.55 Non. Reprod
9712	Jan 5 '15 Apl. 28 '15 113 days	4	18	1	Lf. Ax. M.G to Base Ear Hem. Ad. Carc	10 x 10 x 10 1000 cmm Apr. 10 '15 20 x 15 x 12 3600 cmm.	40 x 40 x 35 56000 cmm	Tumor	Apr 10 - Apr 28 '15	June 10 '14 Apr. 28 '15 322 days	2600 in 95 52400 in 18	27.36 Reprod 2911 11 Non Reprod.
9996	Oct. 24 '14 Mch 31 15 158 days	5	24	1	Rt. Sub - Ax. M.G. Tub. Pap Carc Lungs Replaced Metas	8 x 8 x 8 512 cmm Feb 11 '15 10 x 8 x 8 640 cmm	20 x 15 x 20 6000 cmm	Lung Tumors Retained Fetus Gen. Sepsis	Feb. 11 - Mch. 31 '15	July 7 '13 Mch. 31 '15 632 days	128 in 110 5360 in 48	1.16 Reprod 111.66 Non-Reprod

The youngest of these mice was 282 days, the oldest was 1024 days, the average age was 465 days or 1 year, 3 months, 10 days, the majority of the mice being in the height of the reproductive age at the time of the origin of their tumors. Every mouse from the youngest to the oldest shows a tremendous increase of tumor growth after she ceased to be reproductive.

Note the three cases of mammary gland sarcoma which have been introduced here for purposes of comparison.

1. Female 7454, who had at death a spindle-cell sarcoma right to left axilla 42 x 40 x 30 mm. (50,400 cmm.), grew only 3.35 cmm. of tumor daily during her reproductive period of 217 days after the appearance of tumor, while she grew an average of 1946.88 cmm. of tumor daily in the 25 days she was non-reproductive (Chart 6).

2. Female 8889 had at death a tri-nodular tumor 50 x 30 x 30 mm. (45,000 cmm.), this being a spindle-cell sarcoma between two alveolar tubular carcinomas, the whole growth extending from the left to the right axilla and down to the right forefoot. This mouse showed an average daily tumor growth of 56.91 cmm. during her reproductive period of 123 days, while the rate of growth jumped to an average daily rate of 2643.42 cmm. during the fourteen days she lived after she ceased reproducing (Chart 6).

3. Female 10,015 with a right inguinal alveolar sarcoma and a right axillary partly cystic-squamous, partly papillary adenocarcinoma yielded the following data: The sarcoma grew an average of 1.66 cmm. daily during the 120 days she was reproducing, while an average daily growth of 461.53 cmm. succeeded in the 13 days she was non-reproductive. The carcinoma developed entirely during her non-reproductive period at the daily rate of 15.38 cmm. (Chart 7).

The papillary adenocarcinomas showed the same relative rates of growth during the reproductive and the non-reproductive periods. Note female 7536, (chart 6), average daily growth 0.79 cmm. while reproductive, 77.33 cm. while non-reproductive.

The problem of this retardation of tumor growth during the period of active reproduction is a complex one, and it is necessary to eliminate the other obvious factors in such delay.

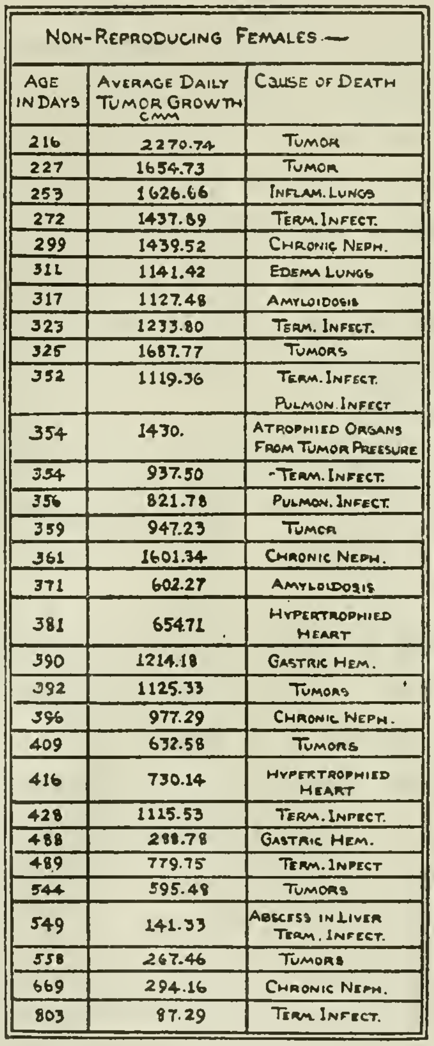
Let me repeat at this point what I already have published frequently, viz: the mice in this laboratory have behind them many generations of hygiene as perfect as the most rigid care can secure. They come of strains whose members are vigorous, sleek, active, well grown, long-lived, and highly reproductive. The individuals taken for this study are among the most vigorous mice in the laboratory. A glance at the number of litters and the number of young they bore after the appearance of their tumors will attest this vigor to anyone who has ever tried the work of breeding mice with spontaneous tumors. These mice have been kept as free from other diseases as it is possible to keep them. Nevertheless many of the cases are complicated by causes of death other than tumor, and this is obviously one of the factors in the varying amount of tumor grown by different individuals. The relation of other diseases to the amount of tumor growth is a subject much too large for the confines of this paper. It will be treated in detail in a forthcoming communication; but let me at this time point out a few facts in this connection as shown by the individuals listed in these charts.

Female 3621 (chart 5) died of an infection characterized by extensive liver necrosis. She lived 22 days after the birth of her last litter (born dead) and grew during this time only 976 cmm. of tumor, 44.36 cmm. daily average. She was 1 year, 1 month, 12 days old, scarcely past the prime of reproductive life in mice. Compare this amount of tumor growth with that of female 8889 (chart 6) who was just 15 days younger and who died of tumors uncomplicated by any other disease. This latter grew 37,008 cmm. of tumor in the 14 days she lived after her last litter, an average daily growth of 2643.42 cmm.

Female 4554 (chart 5) died May 15, 1913, of general sepsis from infected uterus with an undelivered fetus remaining from her last litter, born dead April 30. She was 282 days (9 months, 12 days) old, in the prime of reproductive life. She grew only 784 cmm. of tumor in the 15 days she lived after her last litter, or an average daily growth of 52.26 cmm.

Compare this amount of tumor growth with that of female 9172 (chart 8), fifty-five days older, where the cause of death was not complicated by any other disease than tumor. This latter female grew 99,222 cmm. in the 40 days she lived after the birth of her last litter, an average daily growth of 2480.55 cmm.

CHART 9



NON-REPRODUCING FEMALES—		
AGE IN DAYS	AVERAGE DAILY TUMOR GROWTH CMM	CAUSE OF DEATH
216	2270.74	Tumor
227	1654.73	Tumor
253	1626.66	Inflam. Lungs
272	1437.89	Term. Infect.
299	1439.52	Chronic Neph.
311	1141.42	Edema Lungs
317	1127.48	Amyloidosis
323	1233.80	Term. Infect.
325	1687.77	Tumors
352	1119.36	Term. Infect. Pulmon. Infect
354	1430.	Atrophied Organs From Tumor Preesure
354	937.50	-Term. Infect.
356	821.78	Pulmon. Infect.
359	947.23	Tumor
361	1601.34	Chronic Neph.
371	602.27	Amyloidosis
381	654.71	Hypertrophied Heart
390	1214.18	Gastric Hem.
392	1125.33	Tumors
396	977.29	Chronic Neph.
409	632.58	Tumors
416	730.14	Hypertrophied Heart
428	1115.53	Term. Infect.
488	288.78	Gastric Hem.
489	779.75	Term. Infect
544	595.48	Tumors
549	141.33	Abscess in Liver Term. Infect.
558	267.46	Tumors
669	294.16	Chronic Neph.
803	87.29	Term. Infect.

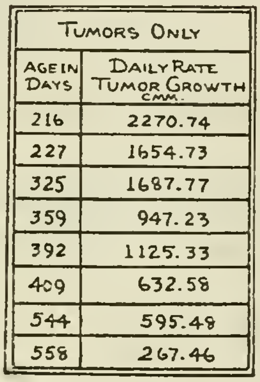
Female 9053 (chart 6), only 293 days old, died of tapeworm. She grew only 548 cmm. of tumor in the 6 days she lived after her last litter, an average daily growth rate of 91.33 cmm.; while female 7536 (chart 6), who died of tapeworm and nematodes, grew an average of only 77.33 cmm. of tumor in the 18 days she lived after her last litter. She was 351 days old.

Compare these two with female 15768 (chart 8), 352 days old, who had only a slight chronic nephritis in addition to her tumors. She produced a daily average of 1194.85 cmm. of tumor in the 20 days she lived after her last litter (born dead); compare also with female 15,990 (chart 7) of about the same age, 348 days, who had a brief acute pulmonary infection. She produced an average of 1448.18 cmm. of tumor daily.

In every tumorous mouse that I have handled whose disease was complicated by tapeworm or nematodes or both, tumor growth has been seriously interfered with, as it has been seriously interfered with by any other rapidly destructive type of complicating disease.

Other diseases, then, are one factor modifying the rate of tumor growth.

CHART 10



Tumors Only	
Age in Days	Daily Rate Tumor Growth cmm.
216	2270.74
227	1654.73
325	1687.77
359	947.23
392	1125.33
409	632.58
544	595.48
558	267.46

Age, as is rather generally admitted, is evidently another factor which modifies the rate of tumor growth.

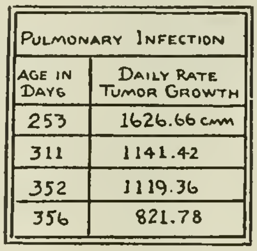
I have drawn up a chart in age sequence, of the thirty females not reproducing after the appearance of their tumors (Chart 9).

The youngest mouse here, 216 days old, showed the largest average rate of tumor growth, viz: 2270.74 cmm. daily, while the oldest mouse, 803 days, produced the smallest daily average of tumor growth, viz: 87.29 cmm. The intermediate mice show a fairly well graduated decrease in average daily tumor growth as the age increases, the relatively few irregularities being explained at least in part by the complicating diseases. For if we take those that died of tumors only, shown in chart 10, the sequence is pretty regular from the youngest, 216 days old and with a daily rate of 2270.74 cmm., to the oldest, 558 days old with a daily rate of 267.46 cmm.

For those dying of a complicating pulmonary infection, the sequence is entirely regular, the youngest, 253 days, growing 1626.66 cmm. daily, while the oldest, 356 days, grew 821.78 cmm. daily.

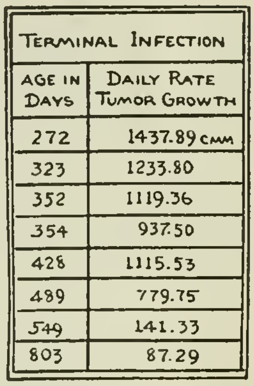
Of those dying of a terminal infection the sequence again is notably regular from the youngest, 272 days, with an average growth of 1437.89 cmm. to the oldest, 803 days, with an average rate of 87.29 cmm.

CHART 11



Pulmonary Infection	
Age in Days	Daily Rate Tumor Growth
253	1626.66 cmm
311	1141.42
352	1119.36
356	821.78

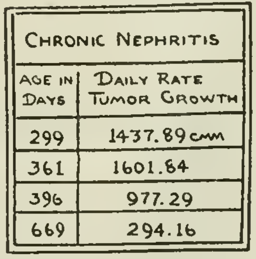
CHART 12



Terminal Infection	
Age in Days	Daily Rate Tumor Growth
272	1437.89 cmm
323	1233.80
352	1119.36
354	937.50
428	1115.53
489	779.75
549	141.33
803	87.29

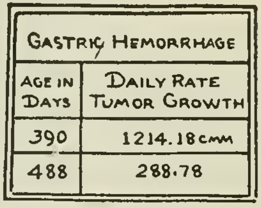
For those dying of chronic nephritis the sequence is fairly regular, and for those dying of gastric hemorrhage, entirely so.

CHART 13



Chronic Nephritis	
Age in Days	Daily Rate Tumor Growth
299	1437.89 cmm
361	1601.84
396	977.29
669	294.16

CHART 14

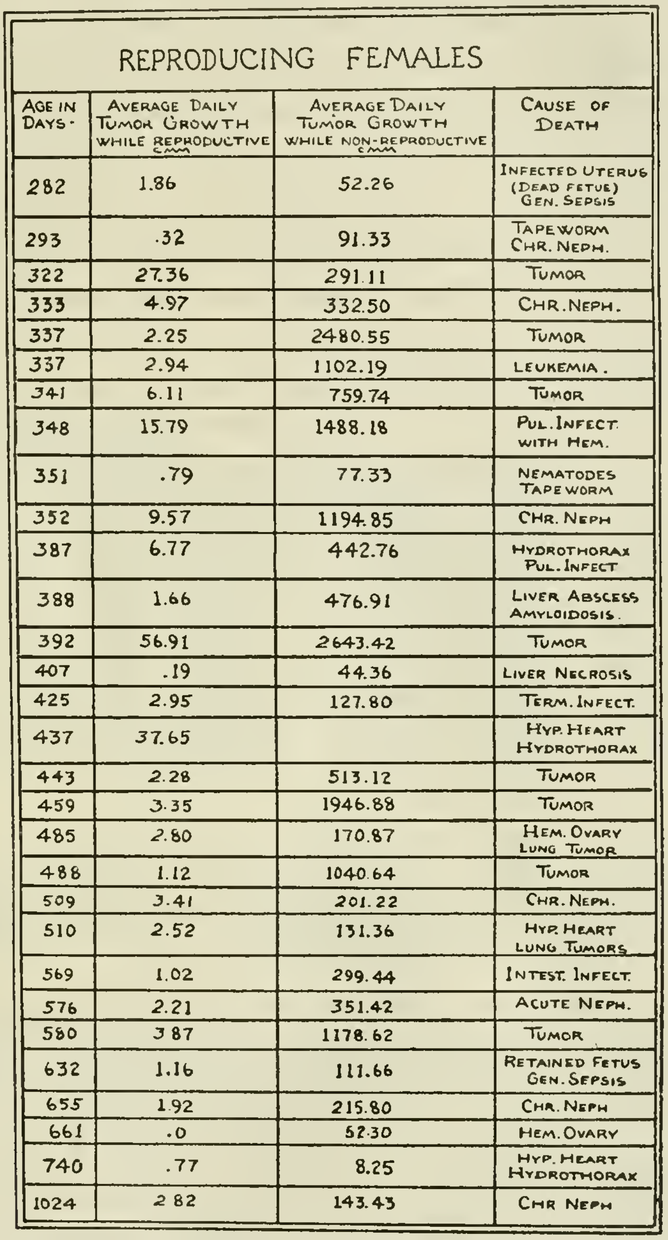


Gastric Hemorrhage	
Age in Days	Daily Rate Tumor Growth
390	1214.18 cmm
488	288.78

In the non-reproducing females, then, there appear only two complicating factors outside of the general metabolic condition of the mouse and the fact that it is at the height of the reproductive age but is not reproducing, viz: the age of the mouse, and those diseases other than tumor which cause death.

Bearing in mind, then, that both age and a destructive complicating disease modify the results, the tremendous amount of tumor uniformly grown by these non-reproducing mice (of reproductive age) stands out with extreme clearness. The normal course of spontaneous tumors in mice of reproductive age which are not being bred, is very rapid. The shortest duration of tumor growth in these mice was 19 days; the longest was 54 days; the average was 35 days, or 1 month, 5 days. These tumors grow to a great size, frequently being as large as the body of the mouse itself. In over 70 per cent of the cases multiple tumors arose, there being but 8 cases with one tumor only; 8 cases with two tumors; 9 cases with three tumors; 4

CHART 15



REPRODUCING FEMALES

AGE IN DAYS.	AVERAGE DAILY TUMOR GROWTH WHILE REPRODUCTIVE cmm	AVERAGE DAILY TUMOR GROWTH WHILE NON-REPRODUCTIVE cmm	CAUSE OF DEATH
282	1.86	52.26	INFECTED UTERUS (DEAD FETUS) GEN. SEPSIS
293	.32	91.33	TAPEWORM CHR. NEPH.
322	27.36	291.11	TUMOR
333	4.97	332.50	CHR. NEPH.
337	2.25	2480.55	TUMOR
337	2.94	1102.19	LEUKEMIA.
341	6.11	759.74	TUMOR
348	15.79	1488.18	PUL. INFECT. WITH HEM.
351	.79	77.33	NEMATODES TAPEWORM
352	9.57	1194.85	CHR. NEPH
387	6.77	442.76	HYDROTHORAX PUL. INFECT
388	1.66	476.91	LIVER ABSCESS AMYLOIDOSIS.
392	56.91	2643.42	TUMOR
407	.19	44.36	LIVER NECROSIS
425	2.95	127.80	TERM. INFECT.
437	37.65		HYP. HEART HYDROTHORAX
443	2.28	513.12	TUMOR
459	3.35	1946.88	TUMOR
485	2.80	170.87	HEM. OVARY LUNG TUMOR
488	1.12	1040.64	TUMOR
509	3.41	201.22	CHR. NEPH.
510	2.52	131.36	HYP. HEART LUNG TUMORS
569	1.02	299.44	INTEST. INFECT.
576	2.21	351.42	ACUTE NEPH.
580	3 87	1178.62	TUMOR
632	1.16	111.66	RETAINED FETUS GEN. SEPSIS
655	1.92	215.80	CHR. NEPH
661	.0	52.30	HEM. OVARY
740	.77	8.25	HYP. HEART HYDROTHORAX
1024	2 82	143.43	CHR NEPH

cases with four tumors; and one case with six tumors. 13 cases, or nearly one-half, showed pulmonary metastases.

In the age chart of the reproducing females the influence of age upon the amount of tumor growth is much less apparent (Chart 15). Even when charted in age periods of 100 days, the influence of age upon tumor growth is much less evident in the reproducing females than in the non-reproducing (Chart 16).

This is in part explained by the complicating causes of death, such as tapeworm and nematodes in the digestive tract, general sepsis from an infected uterus with dead retained fetus, etc. For if we take the females dying of carcinoma only, eliminating those that died of more rapidly growing sarcoma, or of complicating destructive diseases like tapeworm and general sepsis, we get a better sequence (Chart 17).

CHART 16

AVERAGE DAILY GROWTH CMM.

	NON-REPROD.	REPRODUCTIVE	
AGE PERIODS —		NON-REPROD. PERIOD	REPROD. PERIOD
200-300 DAYS	1685.70	71.79	1.09
300-400 DAYS	1108.13	1053.59	12.28
400-500 DAYS	709.35	640.11	7.19
500-600 DAYS	334.75	435.21	2.60
600-700 DAYS	294.16	126.58	.69
700-800 DAYS		8.25	.77
800-900 DAYS	87.29		
1000 DAYS		143.43	2.82

CHART 17

REPRODUCING FEMALES.
DYING OF CARCINOMA ONLY

AGE IN DAYS	TUMOR GROWTH WHILE REPRODUCING	TUMOR GROWTH WHILE NOT REPRODUCING
322	27.36 CMM	2911.11 CMM
337	2.25	2480.55
341	6.11	759.74
443	2.28	513.12
488	1.12	1040.65
580	3.87	1182.00

Even here, however, there is evidence of *some other factor affecting the amount of tumor grown by reproducing females.*

If we chart these same carcinomatous females in sequence of the number of young borne while they were tumorous, we get almost a perfect sequence, the one exception apparently being accounted for by age difference. In these reproducing females, then, *the number of young borne after they are tumorous, seems also to be a factor in determining the amount and rate of tumor growth.*

Bearing in mind these three factors, age, other complicating diseases, and the number of young borne, note the tremendous increase of tumor growth in these mice after they cease reproducing.

The normal course of these spontaneous mammary gland tumors in mice which are constantly reproducing, is very slow. In many cases the tumor scarcely grows at all during this period, one tumor showed no growth whatever during 45 days of reproduction. The lowest daily rate of tumor growth was 0.19 cmm.; the highest daily rate was 56.91 cmm.; the average rate was 7.75 cmm. The duration of the tumor is greatly prolonged by reproduction, the mouse sometimes living nearly a year after the appearance of her tumor while she bears many litters of young. The shortest duration of tumors in these mice was 58 days, the longest 347. The average duration was 177.87 days or 5 months, 27.9 days as compared with 1 month, 6.4 days in the non-reproducing females of about the same age.

CHART 18

REPRODUCING FEMALES.
DYING OF CARCINOMA ONLY

No. of Litters	No. of Young	Rate of Tumor Growth while not reproducing	Age in Days
4	15	2480.55 cmm	337
4	18	2911.11	322
7	20	1182.00	580
7	23	1040.65	488
4	25	759.74	341
8	31	513.12	443

The smallest number of young borne by these mice after their tumor appeared was 7, the largest number was 32; the average was 20.

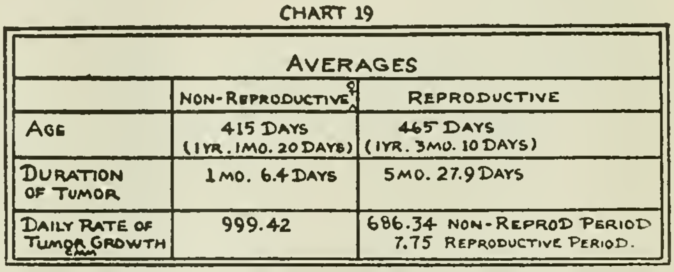
When these females cease reproducing the tumors grow with great rapidity, the mouse frequently living only a few days after the birth of her last litter. The shortest period after the last litter was less than 1 day, the longest was 128 days (in a mouse 2 years, 9 months, 24 days old). The average duration of the tumor in the non-reproductive period was 37 days.

This average duration period is almost exactly the period of duration of the tumor (35 days) in the thirty non-reproductive females of almost the exact age, which therefore make a valuable control in this study of the reproducing females. During this brief period, often only 8 or 10 days, the tumor grows to many times the size attained during the entire period of reproduction, averaging nearly six months.

The lowest daily rate of tumor growth during the non-reproductive period was 8.25 cmm. (in a mouse nearly 2 years old). The highest daily rate was 2911.11 cmm. (in a very young mouse about 10 months old); the average rate was 686.34 cmm., considerably less than the average daily rate of the non-reproducing females of nearly the exact average age (999.45 cmm.) whose tumors were of nearly the exact average duration (35 days).

This emphasizes again the probability that the number of young borne after the appearance of the tumor is a factor in the amount of tumor grown after the reproductive period is past, and links these two modes of growth, the growth of embryos and the growth of tumor in a very close relationship. Multiple tumors appeared much less frequently in these reproducing females than in the non-reproducing. There were 15 cases of single tumor growth, as compared with 8 cases in the non-reproductive females. There were 9 cases with two tumors, as compared with 8 in the non-reproducing females, 5 cases of 3 tumors as compared with 4 in the non-reproducing, while 4 was the highest number of tumors occurring in any one of these reproducing mice, there being but one such case. 50 per cent of these cases showed multiple tumors as compared with over 70 per cent of multiple tumors in the non-reproducing females. Ten cases, or one third, showed lung metastases.

CHART 19



AVERAGES		
	NON-REPRODUCTIVE ♀	REPRODUCTIVE
AGE	415 DAYS (1 YR. 1 MO. 20 DAYS)	465 DAYS (1 YR. 3 MO. 10 DAYS)
DURATION OF TUMOR	1 MO. 6.4 DAYS	5 MO. 27.9 DAYS
DAILY RATE OF TUMOR GROWTH CMM	999.42	686.34 NON-REPROD PERIOD 7.75 REPRODUCTIVE PERIOD.

Chart 19 shows the average age, duration of tumor growth, and rate of tumor growth of the 2 sets of mice, the non-reproducing and the reproducing.

SUMMARY

In handling large numbers of mice with spontaneous tumors there is forced upon the observer from the very first the great difference in the rate of tumor growth in the non-reproducing and in the reproducing females.

The same difference is noted in the rate of tumor growth in the non-reproductive and in the reproductive periods of the same female.

For this study thirty each of non-reproducing and of reproducing females with spontaneous tumors were selected. The tumors were all of the same type and of the same organ (with a few exceptions for purposes of comparison), viz: alveolar tubular carcinoma of the mammary gland, of which a daily observation is easily made.

Without exception, the amount of tumor grown by a female while reproductive was strikingly less than during her non-reproductive period.

Again, the amount of tumor grown by reproducing females was strikingly less than that grown by non-reproducing females.

The normal course of these spontaneous tumors in mice that are not bred is very rapid, the mouse rarely living over six weeks and often less than a month after the appearance of her tumor. The tumors grow to a great size, frequently being as large as the body of the mouse.

When, however, these tumorous mice are bred, the tumor scarcely grows at all during the reproductive period. The duration of the tumor is greatly prolonged, the mice frequently living nearly a year after the appearance of their tumors, during which time many bear from six to eight litters aggregating from twenty to thirty-two young. When the mouse ceases reproducing, the tumors grow with tremendous rapidity and to great size, the female frequently surviving only six or eight days after the birth of the last litter. During this brief period the tumor grows to many times its size at the date of the last litter.

In brief, during the six or eight days a mouse is non-reproductive, she grows enormously more tumor than during the eight months or a year while she is reproductive, the daily rate of tumor growth being far in excess of the daily rate during the reproductive period.

Two other factors must be taken into consideration, viz: the age of the mouse, and other complicating causes of death. Generally speaking, the younger mice show a higher daily rate of tumor growth than do the older mice.

Again, complicating diseases such as tapeworm and nematodes in the digestive tract, or any other highly destructive disease, greatly retard tumor growth; and *the number of young borne after the appearance of tumor is also a factor in reproducing females.* But when these factors have been eliminated two facts stand out with startling clarity and cannot be gainsaid, viz:

1. Reproducing females grow much less tumor than do non-reproducing females of the same approximate age and general metabolic condition.

2. Reproducing females grow much less tumor while they are reproductive than they do while they are non-reproductive; in other words, *when a mouse is producing embryos, she is not producing tumor in anything like the amount which she grows while non-reproductive.* Multiple tumors are more common in the non-reproducing than in the reproducing females, the figures being over 70 per cent compared with 50 per cent.

In striking contrast to these results is the relation between pregnancy and the infections common to mice. If an infected mouse is bred, instead of having the infection held off for a year or more while she bears young, she is unable to produce any young at all and speedily dies of her infection. Or if a pregnant mouse contracts an infection, she rarely brings her young to birth.

The results of this study bring out with striking force the close relation between tumor production and the production of young, showing them to be two closely related modes of growth.

CONCLUSIONS

1. Cancer and reproduction, both being growth processes, draw upon the same energy residuum and are made possible by the same food. Hence the food and energy used by one are withheld from the other.

2. Therefore (a) if the female is *constantly pregnant,* energy and food are withheld from the tumor and it grows with extreme slowness. (b) If there is a hiatus between pregnancies, or a termination of pregnancy, the energy which was running into reproduction is released and diverted into tumor which grows very rapidly. (c) If tumor growth considerably antedates impregnation, the currents of energy are already being used for tumor growth and are with difficulty diverted for pregnancy, probably never wholly so.

3. Hence, when a female is well advanced in tumor growth before impregnation there are rarely any offspring brought to birth. When offspring are delivered they are few, small, undernourished, and rarely suckled (which in mice means there is no lactation).

4. When tumor growth is not interfered with by pregnancy, it is (a) extremely rapid in mice which are young, well nourished, and vigorous; (b) less rapid in mice older or less vigorous, or less nourished; (c) very slow in mice which are old, feeble, under nourished, or afflicted with a destructive complicating disease.

5. Another point which shows the close relation between the growth of embryos and the growth of tumor is the great frequency with which breast tumors are nearly synchronous with delivery. Hyperstimulation of any tissue seems to originate cancer of those tissues in individuals of cancer tendency; hence the intense stimulation incident upon lactation tends to originate cancer of the breast in individuals of breast cancer tendency.[3]

6. The prolonged hiatus between pregnancies greatly complicates the study of the relation between pregnancy and tumor growth in the human species. During this prolonged hiatus the tumor may draw off the energy which would have continued to be used in reproduction if the pregnancies were not widely separated, just as is the case in mice kept constantly impregnated. This would account for any apparently conflicting testimony in human experience as compared with these studies.

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[3] This subject will be more fully treated in a forthcoming paper.

The factors are not subject to control in the attempt to study the relation between reproduction and tumor growth in the human species, and the conclusions have to be drawn without knowledge of complicating factors. The real relation between these two can be disclosed only in the experimental laboratory, where the factors are all known and are under control.

The experimental evidence shows a very striking relation between these two modes of growth, the production of young and the production of tumor; moreover, it shows the same relation between the production of young, and the growth of *all types* of mammary gland tumors.

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THE RELATION OF INBREEDING TO TUMOR PRODUCTION: STUDIES IN THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE

XIII. PROBLEMS IN THE BEHAVIOR OF TUMORS

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Throughout the range of cancer research, there seems to be no other point so frequently and so completely misunderstood as the subject of inbreeding in its relation to the incidence of tumor. Since the beginning of this series of publications, these researches into the problem of the inheritability of cancer have been met by the statement that "inbreeding increases the number of tumors in a strain," or that "inbreeding is responsible for the high incidence of tumors in a strain of mice, and consequently the demonstration of the inheritability of cancer for mice has no bearing upon the human species, since the latter is not characterized by inbreeding."

The latest and most conspicuous example of this misapprehension of biological procedure and fact appears in Ewing's recent compendium "Neoplastic Diseases," (1) in which he states that "Bashford *attempted* by inbreeding to *intensify* the hereditary influence," and that "Slye *has proven* that *inbreeding* of *tumor-bearing animals greatly increases* the *incidence* of tumors." With this misinterpretation, he dismisses all the exact indisputable experimental evidence for the inheritability of tumors in general and of all types, including cancer, in particular.

It is precisely because inbreeding does not characterize the human species that it is impossible to make any even reasonably complete or accurate study of the inheritability of cancer in that species, and hence that experimental evidence becomes absolutely necessary as it is impossible to *prove* the inheritability of any character without inbreeding. Mendel in his work with peas was not trying to increase roundness or ovalness, yellowness or greeness, tallness or shortness, or any other quality of peas. He was trying to find out whether these characters were hereditary, and in order to find out whether they were hereditary, he *had to inbreed his peas.*

Cuenot (2) when he crossed the albino mouse with the house mouse, was not trying to increase albinism or greyness or any other quality of mice, he was trying to find out whether pigmentation and lack of pigmentation are hereditary; and in order to do this he *had to inbreed his mice.* He did not thereby *increase* albinism, or *increase* the agouti coat, or *increase* any other character. He demonstrated that pigmentation and albinism are inheritable and in demonstrating that pigmentation and lack of pigmentation are inheritable in mice (whose pigment is melanin) he demonstrated that these characters are inheritable also in guinea pigs, or in rabbits, or in man (in all of which species the pigment is melanin).

Neither Bashford nor Slye has "*attempted to prove,*" or "proved that inbreeding increases the incidence of tumors." Neither have Lathrop and Loeb or Little and Tyzzer attempted to demonstrate any such absurdity. All of these experimental workers in cancer have attempted to prove or disprove the inheritability of cancer and they have *all inbred their animals*, because only by inbreeding can you analyze a strain. By inbreeding one discovers what is in a strain, *he does not "increase" or "intensify" anything.*

For the last ten years there have appeared from this laboratory many charts showing the exact genealogical data of strains of mice (families) in which cancer has been transmitted through generation after generation without a break and *in strikingly perfect Mendelian ratios.* At the same time other charts have been published showing the exact genealogical data of families of mice in which there has never been any tumor of any sort, through generation after generation, where the mice have been handled with identical technique *including constant inbreeding.*

The genealogical charts which have appeared in previous reports from this laboratory have all involved the inbreeding of filial generations of offspring from hybrid crosses, because only so is it possible to find out accurately *just what characters are transmitted in a hybrid cross* and *in just what ratio they are transmitted.* This is the method of all biological procedure in the study of heredity. There is no other method. Just as the analytical chemist does not analyze his unknown by throwing in more unknowns, so the analytical biologist cannot analyze his strain by throwing in more unknowns (by hybridizing) but must inbreed. For the benefit, however, of those who do not comprehend or accept this method, I have drawn up a chart of matings made in this laboratory, which is the duplicate of human mating, and in order that no point in it may escape observation I have decorated it with human nomenclature (Chart 1).

This chart is the exact duplicate of human genealogical charts, in that it contains no inbreeding and not even any consanguineous matings. Miss Smith (female 1) is mated with Mr. Jones (male 167); the offspring of this mating, viz., Miss Jones (female 791) is mated with young Mr. Ham (male 7612) whose medical genealogy is indicated in the chart; etc. Through seven generations one daughter of each pair, is in turn mated with a wholly unrelated male, whose medical genealogy is shown. Every mating in the chart *is a hybrid cross,* just as is the case in all human matings.

Note (1) that carcinoma of the mammary gland entered in the parent female who began this family, viz. Miss Smith (female 1); (2) that the male parent in each generation was the immediate offspring of a cancerous progenitor; (3) that the daughter in every generation exhibited carcinoma of the mammary gland; (4) that lung tumors entered on the male side in the fifth generation; (5) that these lung tumors were exhibited by each succeeding generation (generations 6 and 7).

It must not be inferred from this chart that mammary gland carcinoma can be transmitted only by the female, or lung tumor only by the male. They happened to be so transmitted in this family. But there are many other families in which mammary

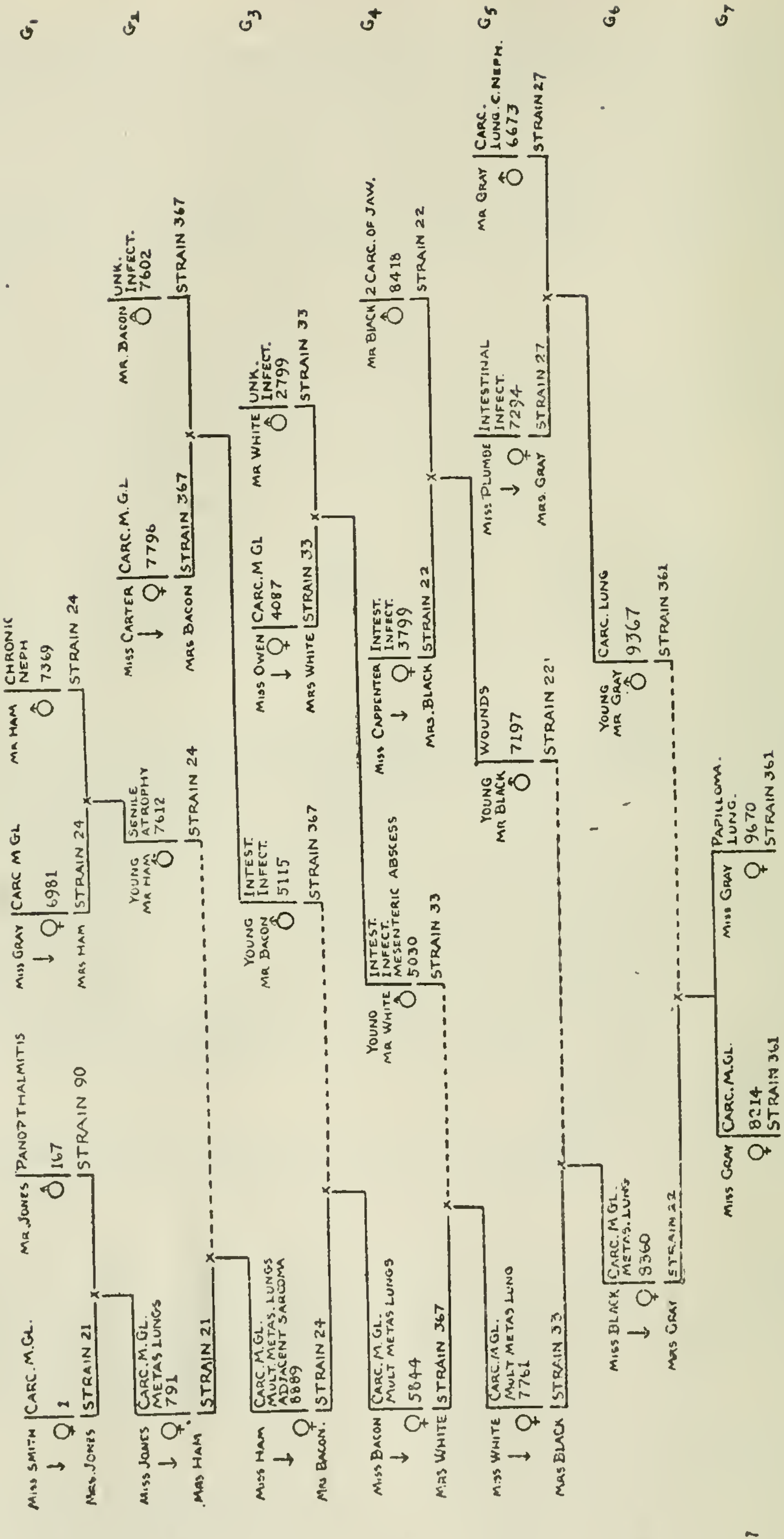

G1
Miss Smith ↓ Mrs. Jones
Carc. M.Gl. ♀ 1
Strain 21
Mr Jones ♂ 167
Panopthalmitis
Strain 90
Miss Gray ↓ Mrs Ham
Carc M Gl ♀ 6981
Strain 24
Mr Ham ♂ 7369
Chronic Neph
Strain 24
G2
Miss Jones ↓ Mrs Ham
Carc. M. Gl. Metas Lungs ♀ 791
Strain 21
Young Mr Ham ♂
Senile Atrophy 7612
Strain 24
Miss Carter ↓ Mrs Bacon
Carc. M. G. L ♀ 7796
Strain 367
Mr. Bacon ♂
Unk. Infect. 7602
Strain 367
G3
Miss Ham ↓ Mrs Bacon
Carc. M. Gl. Mult. Metas. Lungs Adjacent Sarcoma ♀ 8889
Strain 24
Young Mr Bacon ♂
Intest. Infect. 5115
Strain 367
Miss Owen ↓ Mrs White
Carc. M Gl ♀ 4087
Strain 33
Mr White ♂
Unk. Infect. 2799
Strain 33
G4
Miss Bacon ↓ Mrs White
Carc. M. Gl. Mult Metas Lungs ♀ 5844
Strain 367
Young Mr White ♂
Intest. Infect. Mesenteric Abscess 5030
Strain 33
Miss Carpenter ↓ Mrs. Black
Intest. Infect. ♀ 3799
Strain 22
Mr Black ♂
2 Carc. of Jaw. 8418
Strain 22
G5
Miss White ↓ Mrs Black
Carc. M. Gl. Mult Metas Lung ♀ 7761
Strain 33
Young Mr Black ♂
Wounds 7197
Strain 22'
Miss Plumbe ↓ Mrs. Gray
Intestinal Infect. ♀ 7294
Strain 27
Mr Gray ♂
Carc. Lung. C. Neph. 6673
Strain 27
G6
Miss Black ↓ Mrs Gray
Carc. M Gl. Metas. Lungs ♀ 8360
Strain 22
Young Mr Gray ♂
Carc. Lung 9367
Strain 361
G7
Miss Gray
Carc. M. Gl. ♀ 8214
Strain 361
Miss Gray ♀
Papilloma. Lung. 9670
Strain 361

CHART I

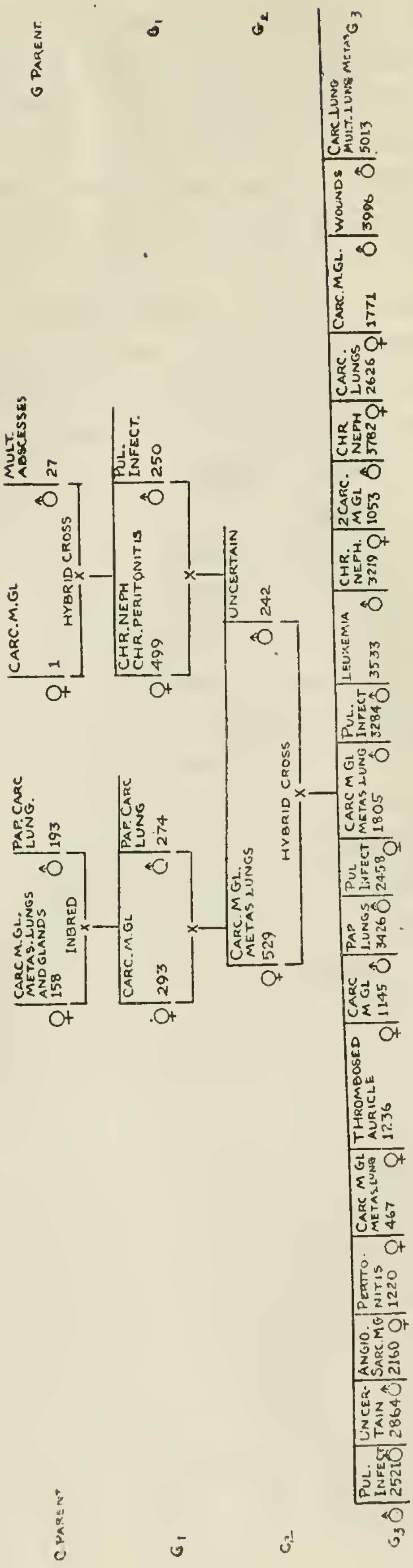
gland carcinoma was transmitted by the male (note chart 2, strain 146, branch II) and lung carcinoma by the female. Throughout the work of this laboratory no allelomorphism has been demonstrated between sex and type or location of tumor, except, of course, in the sex organ tumors. There is not apparently any allelomorphism even between sex and mammary gland tumors, since this laboratory has yielded a considerable number of males with mammary gland tumors and males who have transmitted mammary gland tumors. The great preponderance of this location of tumor in the female is presumably due to the greater frequency of hyperstimulation of these tissues in the female. Loeb (3) suggests the possibility that the female may be more potent than the male in the transmission of mammary gland cancer. This suggestion is not borne out by the results in this laboratory, wherein the female is no more prepotent in the transmission of mammary gland carcinoma than she is in the transmission of any other character; and the male is just as potent to transmit mammary gland cancer as he is to transmit a grey coat-color. His female offspring demonstrate the inheritance of mammary gland cancer more frequently than his male offspring for the reason stated above, viz., the more frequent chronic irritation of mammary gland tissues in the female. This subject will be discussed more fully in a forthcoming paper.

Innumerable matings of this sort (i.e. like human matings) can be made and their charts published, but it is useless to waste many cancerous mice in such crosses, because they are impossible of accurate analysis. It is best to follow the exact method whereby through analysis one can become acquainted with the intrinsic characters of individual mice and of strains of mice. It is then possible to manipulate the characters of the individual mice or of the strains of mice with a certainty of outcome; entirely eliminating the uncertainty attendant upon the so-called "statistical method," which in reality is not a method at all.

Let us analyze, therefore, some of the charts which have been misunderstood to demonstrate that "inbreeding increases tumor." These charts have been published in reports from this laboratory to which Ewing refers (4), (5), (6). Chart 2 shows the first filial

CHART 2

STRAIN 146 WITH ANCESTRY.



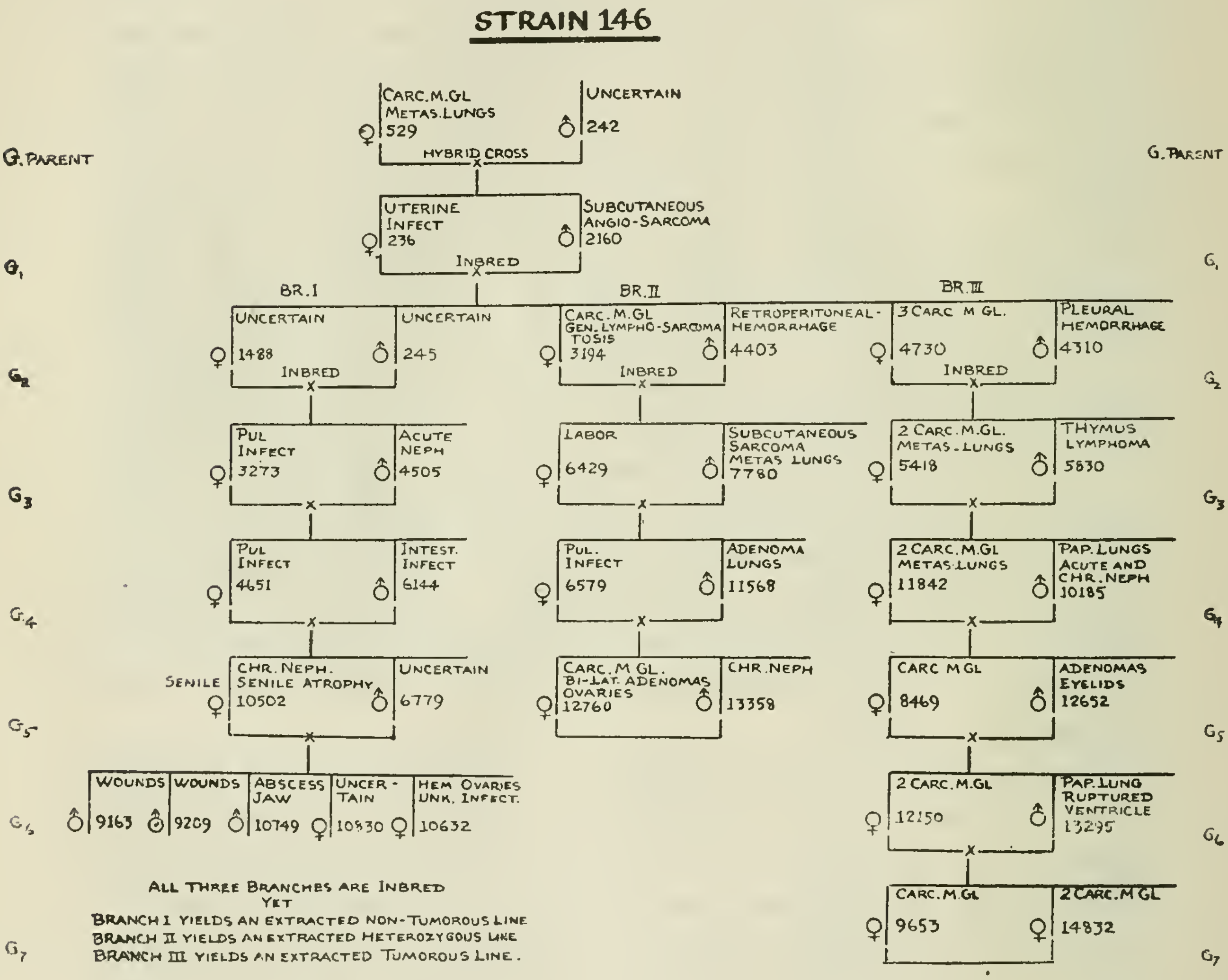
generation of strain 146, with its ancestry for three generations. This strain is the product of a *hybrid cross*, female 529 being in no way related to her mate, male 242. Female 529 died of carcinoma of the mammary gland with metastases in the lungs. Her mother and her grandmother, females 293 and 158, both died of carcinoma of the mammary gland. Her father and her grandfather, males 274 and 193, both died of carcinoma of the lung. She came of a family which carried in all its branches 100 per cent of carcinoma. Her ancestry for two generations was inbred, and we therefore know her to be an extracted cancer-bearing individual, capable of carrying cancer into any strain into which she is mated, with the same certainty with which she will carry albinism. We have then in her, by this method of inbreeding, not an unknown factor, but one whose influence in any compound of which she forms a part can be predicted both in the matter of color and of disease; and whose influence has actually been demonstrated to be just as predicted, both in regard to albinism and in regard to cancer. Her mate, male 242, died of an undetermined disease and had no tumor. His father and his mother, male 250 and female 499, did not exhibit tumor of any sort; his grandmother, female 1, however, died of carcinoma of the mammary gland; his grandfather, male 27, of multiple abscesses without tumor. He comes, then, of a *hybrid*, not an inbred line, with carcinoma of the mammary gland two generations back.

His mating with female 529 is a *hybrid cross* (*not inbred*). Note the number of tumorous individuals in the first *hybrid* generation, viz., nine out of nineteen (of the young who lived to cancer age), the almost exact Mendelian expectation from the mating of an extracted cancerous female with a male heterozygous to cancer (that is carrying it potentially, but not himself developing the disease). A hybrid strain then, which carries 47 per cent of tumor in its first generation (*without inbreeding*) is the product of this cross.

Chart 3 carries on three branches of this hybrid strain 146, and shows what results followed *inbreeding* in each of the above branches.

We have here this same female 529, an extracted cancer-bearing female, mated with male 242 (a heterozygote). The two first-generation hybrids mated in this chart are female 236, a non-cancerous female who died of uterine infection, and male 2160 who died of a subcutaneous angiosarcoma, probably of the mammary gland. Branch I, *although closely inbred* (brother and sister from the same litter in each generation) never yielded a case of any kind of tumor whatever, *i.e.*, it is an extracted non-tumorous line. Branch II, also closely *inbred in exactly the same way* as branch I, yielded a heterozygous line, the tumors being transmitted sometimes through the male and

CHART 3



STRAIN 146

ALL THREE BRANCHES ARE INBRED
YET
BRANCH I YIELDS AN EXTRACTED NON-TUMOROUS LINE
BRANCH II YIELDS AN EXTRACTED HETEROZYGOUS LINE
BRANCH III YIELDS AN EXTRACTED TUMOROUS LINE.

sometimes through the female. (Note the mammary gland tumor transmitted through the male in branch II.) While branch III, also *inbred in exactly the same way* as branches I and II, yielded an extracted tumorous line, nearly 60 per cent of these tumors being carcinoma of the mammary gland.

Here are three branches then of the same hybrid family from a cancerous mother, all *inbred in exactly the same way*, yielding three totally different results, *thereby completely eliminating inbreeding as a determining influence* in the incidence or the ratio of tumor production. Moreover, these results are exactly in accordance with Mendelian law.

Ewing (7) states that "the Mendelian characters noted in the heredity of some pathological conditions have not been traced with tumor." All the charts in this paper, and all other charts put out from this laboratory clearly show the "Mendelian character of tumor inheritance." He states further: "the predisposition might be congenital without being hereditary." It is an axiom in genetics that *characters which segregate out*, as do cancer and non-cancer, are hereditary and not congenital. If we do not admit this, no character has ever been proved to be hereditary.

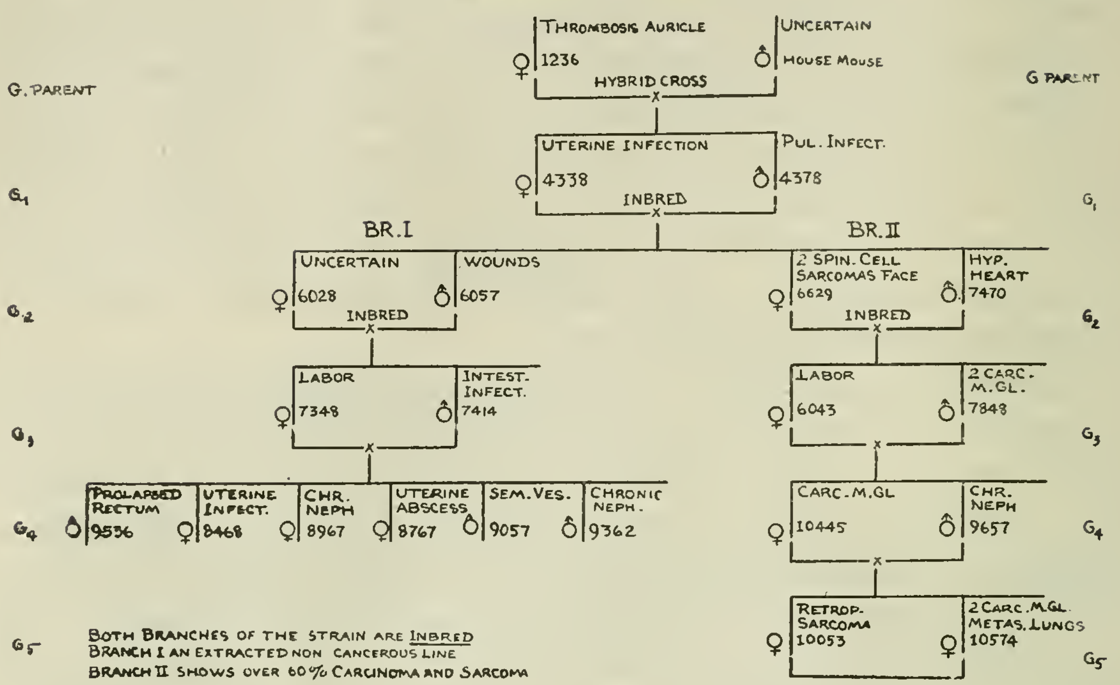
Chart 4 shows strain 164, branch IV, carried out in two families. This is a *hybrid* strain whose progenitors were in no way related. The parent male was a common house-mouse of a strain in my hands many years with no trace of tumor of any sort, although it was *consistently inbred.*

The parent female was a first generation heterozygote of strain 146 (shown in chart 2) who carried cancer into every strain of which she was progenitor, although she herself did not exhibit cancer.

The strain is here shown in two branches *both rigidly inbred.* Branch I is an extracted non-cancerous line, whose members, neither in inbreeding nor in hybridization, ever produced a cancerous offspring. That is, they are proved non-cancerous individuals, whose influence can be predicted in every family into which they are introduced.

CHART 4

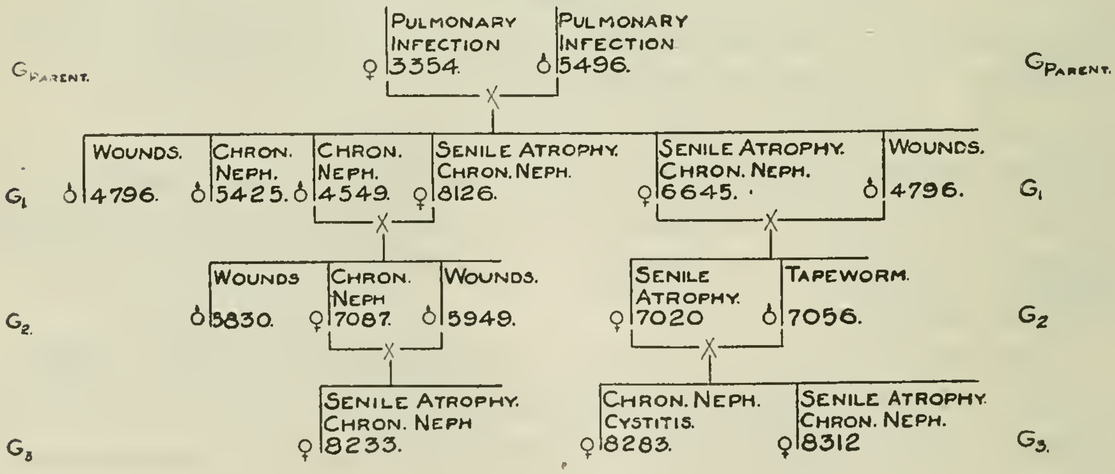
STRAIN 164 BRANCH IV



BOTH BRANCHES OF THE STRAIN ARE INBRED
BRANCH I AN EXTRACTED NON CANCEROUS LINE
BRANCH II SHOWS OVER 60% CARCINOMA AND SARCOMA

CHART 5

STRAIN 20.



A NON-CANCEROUS STRAIN OF JAPANESE WALTZING MICE
BOTH PARENTS DIED OF PULMONARY INFECTION
THE RESULTING STRAIN SHOWS 0% PULMONARY INFECTION.

Branch II *inbred in exactly the same way* yields over 60 per cent of carcinoma and sarcoma and carries both of these types of neoplasms into every strain into which they are introduced. *Analyzed individuals, then, whose influence can be predicted, are the outcome of the inbreeding method.*

Chart 5 shows strain 20 which has been *closely inbred*, both in the original strain and in its hybrid derivatives, for ten years in my hands, without displaying a tumor of any kind. It is shown in this chart through four generations only; but they are typical of the entire strain and its derivatives, in none of which has cancer or any other tumor ever appeared although the strain has been rigidly inbred.

The foregoing charts are typical. They show conclusively that *inbreeding is not a factor in the increase of tumor*, or in the determination of its incidence; it is merely a method of analyzing a strain in order to determine whether that strain carries cancer or whatever character may be under study.

The real effect of inbreeding upon tumor production seems wholly to have escaped those critics like Ewing who attribute to it any increase in tumor production, although this point was discussed at some length in the Third Report from this laboratory published in March, 1915. It seems advisable, therefore, at this time to demonstrate again as clearly as possible just what is this influence of inbreeding. (Note chart 6, strain 139.)

The parents, female 158 and male 193 (both with carcinoma), were the product of many generations of close inbreeding. They produced only five offspring (compare this with the output of any hybrid cross). These five offspring coming of double cancerous parentage were all cancerous. Four were males and only one a female.

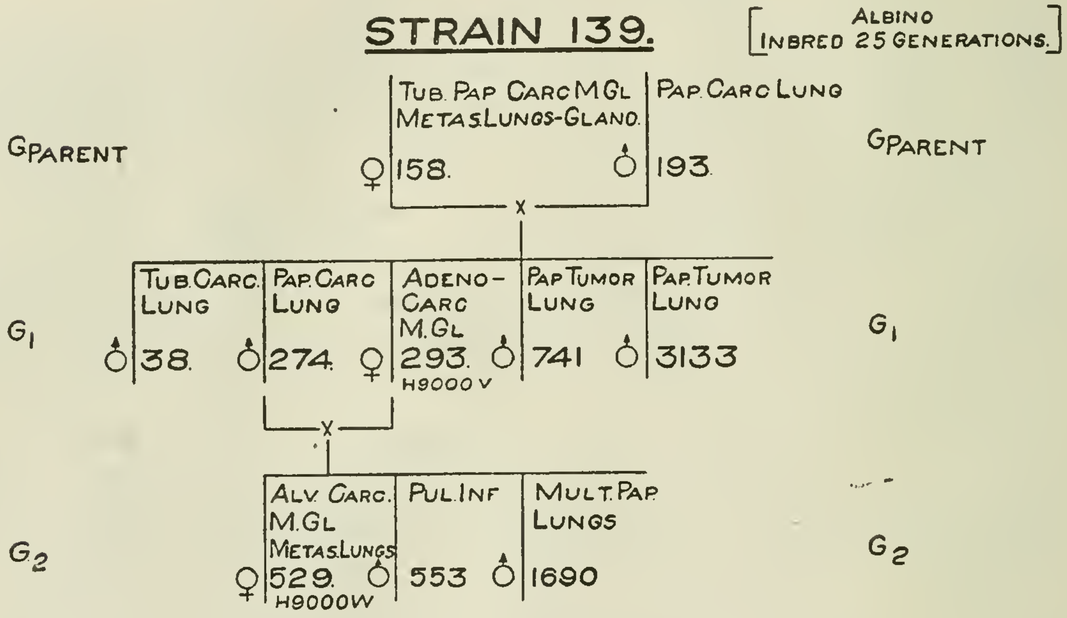
The mating of this one female, number 293, with her brother male 274 (both with carcinoma) produced only three young, two males and one female, all tumorous except the one who died under cancer age, viz., male 553.

The mating of female 529 with her brother male 1690, produced no offspring whatever, and the strain therefore *through close inbreeding was completely eliminated.* Elimination, then, and not increase in tumor, was the result of this inbreeding.

When, however, female 529 who had no young by her brother, was *hybridized* with male 242 (totally unrelated) she became the progenitor of an extremely prolific strain, into which she carried both cancer and albinism. This strain persists in the laboratory to this date, and has in turn furnished the progenitors of many prolific hybrid cancerous strains. This strain, 146, is the one some of whose branches are shown in charts 2 and 3 in this paper.

Again, female 293, first filial generation in strain 139 (chart 6) mated with her brother, male 274 (both cancerous) produced only three young; mated in turn with her other brothers, male 38 and male 741 (both cancerous) she produced no young. When, however, she was hybridized with male 25 from a totally unrelated strain, she started up a strong prolific line, strain 65, carrying into it both cancer and albinism (shown in two branches in chart 7). Here, again, it was *hybridization which increased the production of tumor; inbreeding which eliminated tumor.*

CHART 6

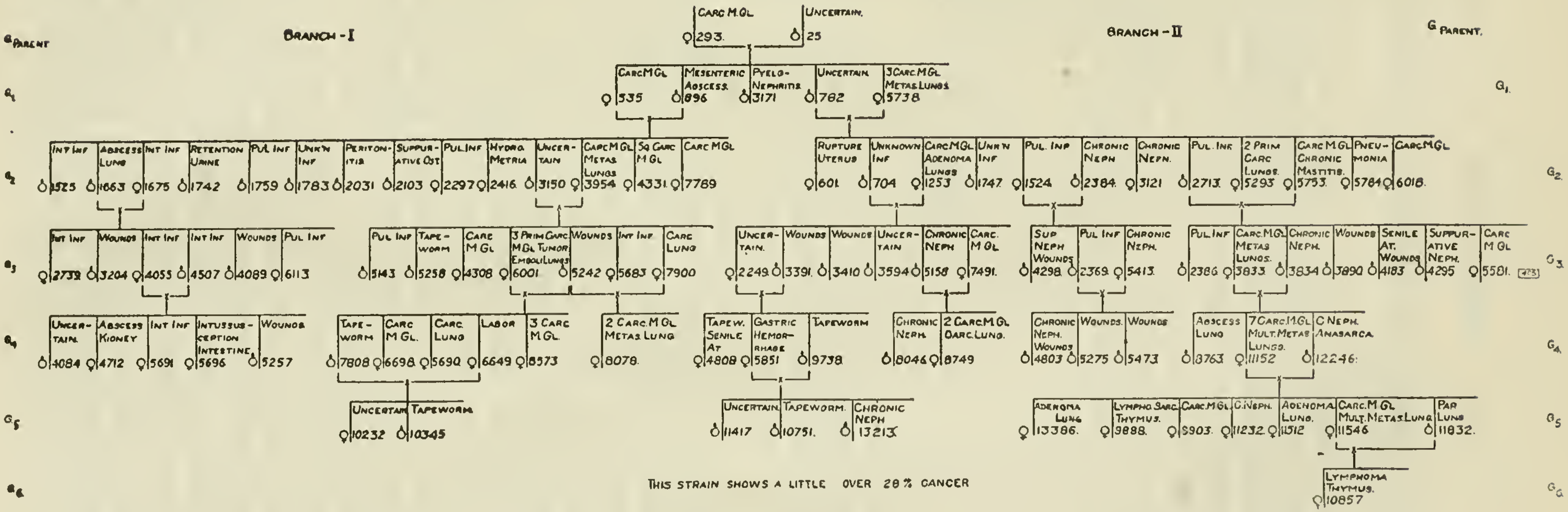


THIS STRAIN SHOWS 90% CANCER.

Instances of this sort could be multiplied in any number desired, but the cases shown in the foregoing charts are entirely typical. *Racially therefore, inbreeding eliminates tumor.*

CHART 7

STRAIN-65.



Parent ♀Strain 139
Parent ♂Strain 90

This strain shows a little over 28% cancer

The literature yields very little on the subject of inbreeding which is of interest here.

Lathrop and Loeb (9) state: "Continued study of strains of mice in which we have established the tumor rate for earlier generations shows that in the majority of cases the rate remains the same throughout later generations. In most of these strains the constancy in the tumor rate is striking. In a few exceptional cases the rate has increased, but in a considerable number of strains there is a distinct fall." This statement, as well as the explanations offered, indicates that the method was, in the main, mass breeding within the stock and not the controlled selective breeding of individuals. The fall or rise of the tumor rate or its static condition within a strain, would thus be attributable to the reasons assigned by the authors or to any one or combination of many other reasons.

Only by the controlled inbreeding of definite individuals, is it possible to eliminate all other influences determining the rise or fall of the tumor rate. Throughout the work of this laboratory the word *strain* has been used to signify a family arising from a mating between two definite known individuals of known ancestry and known cause of death, the subsequent matings within the strain also being between definite, known individuals of known cause of death. Lathrop and Loeb use the word *strain* as this laboratory uses the word *stock*. These authors say further, "As the result of long continued inbreeding, certain characteristics of a strain change. The strain becomes less prolific and less vigorous, and hand in hand with this change goes a lowering of the tumor rate. This occurred in strain 8 and possibly in other strains."

What these authors find to be true of only one strain, I find to be the law in all strains derived as explained above from a single pair, with all later generations derived from the offspring of this one pair, without the introduction anywhere in any generation of any other member even of the same original stock. This method of mating is the only real inbreeding and the only accurate method of testing the effect of inbreeding. Where this method is followed the most complete and exact analysis possible is made of the characters transmitted by the original pair.

In the Third Report of this series, published in March, 1915, I stated, "Inbreeding if persisted in eventually wipes out any strain which I have handled" (8). This statement remains unqualified to the present date, there never having been an exception to the rule.

The instances cited in this paper are perfectly typical of all of the hundreds of strains handled in this laboratory. This test has been made consistently for twelve years, and from the facts we are justified in drawing this conclusion: *Consistent inbreeding eliminates any strain.*

It has been the pet argument of workers who maintained that inbreeding had no deleterious effect on a strain, that if perfect individuals were selected for the matings no weakness would be transmitted. A pretty theory but wholly opposed to the facts; for if there were these "perfect individuals" some matings of them would have occurred, and in such strains we should have achieved immortality.

The length of time it takes to eliminate a strain by close inbreeding will depend (1) upon whether or not the stocks crossed produce a fertile hybrid stock. For example, the fancy stocks derived in this laboratory from Mus musculus do not produce a prolific hybrid stock when mated with the Japanese Waltzing mouse. Neither does Mus musculus itself produce a prolific stock when mated with the Japanese Waltzer. Many such strains have been produced in this laboratory, no one of which has ever matched in fertility or vigor of progeny the hybrid strains produced by crossing different stocks where both were Mus musculus derivatives. It will depend, also, (2) upon the vigor of the original pair and whether or not they carry any of the same weaknesses or defects; and (3) upon the vigor of each succeeding pair and what same weaknesses and defects they carry, since they are certain to carry some of the same defects.

If either the original pair or any succeeding pair both carry general defects or weaknesses of the respiratory tract, or of the digestive tract or any organ of this tract, or of the circulatory tract, etc., the strain will quickly run out, since the offspring receive a double dose of this weakness and inevitably transmit it to all offspring. If both original parents or both parents in any succeeding generation carry cancer or any other type of tumor, the strain will be eliminated relatively quickly, since cancer or any other type of tumor interferes with prolific reproduction (10) and since the offspring receive a double dose of cancer and inevitably transmit it.

Inbreeding then, within a cancer strain, speedily eliminates the strain and instead of increasing cancer as some have inferred, eliminates cancer.

This infertility and general inferiority of the strains derived from crossing the Japanese Waltzer with Mus musculus and its derivatives undoubtedly explains in considerable part if not wholly the results of Little and Tyzzer (11) in their studies in the inheritability of tumor "takes" of their implanted carcinoma, J. w. A., in hybrids of Japanese Waltzers and Mus musculus and its derivatives.

The very fact that J. w. A. does not "take" in Mus musculus or its derivatives, indicates a marked difference in these two races. This same marked difference militates also against a vigorous and prolific strain from the hybridization of these two races.

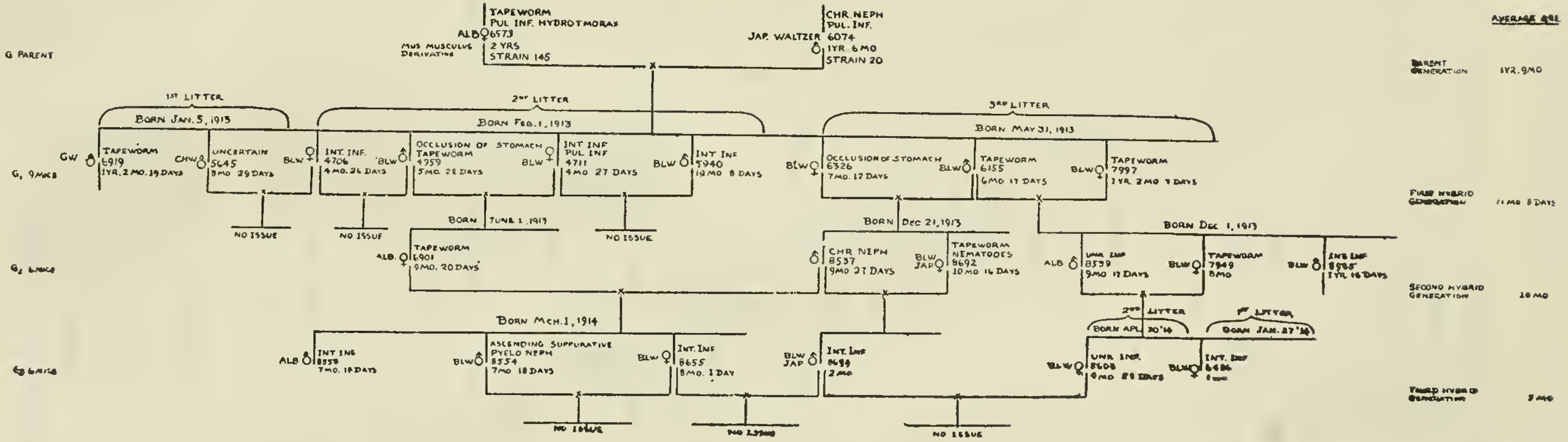
In this laboratory many such crosses have been made. In every case the first generation hybrids have been apparently vigorous mice, inheriting their size from Mus musculus derivatives. But when inbred, they have yielded a very meager output of second generation hybrids, and in no case has it been possible to carry these strains beyond the third hybrid generation, rarely beyond the second. The mice of hybrid generations later than the second are small and apparently entirely infertile when inbred; they are also short lived and relatively inactive.

Chart 8 shows one of these families, strain 479, which is perfectly typical of all the rest.

Strain 479 was produced by mating a Mus musculus derivative, albino female 6573 of strain 145, with Japanese Waltzer male 6074 of strain 20. Female 6573 was a vigorous prolific

CHART 8

STRAIN 479


G PARENT
G1 9 MICE
G2 6 MICE
G3 6 MICE
TAPEWORM
PUL INF. HYDROTMORAX
ALB ♀ 6573
2 YRS
STRAIN 145
MUS MUSCULUS DERIVATIVE
CHR. NEPH
PUL. INF.
JAP. WALTZER ♂ 6074
1 YR 6 MO
STRAIN 20
1ST LITTER
BORN JAN. 5, 1913
GW ♂ TAPEWORM 6919 1 YR. 2 MO. 19 DAYS
CHW ♂ UNCERTAIN 5645 8 MO 29 DAYS
BLW ♀ INT. INF. 4706 4 MO. 26 DAYS
NO ISSUE
NO ISSUE
2ND LITTER
BORN FEB. 1, 1913
BLW ♂ OCCLUSION OF STOMACH TAPEWORM 4959 5 MO. 28 DAYS
BLW ♀ INT INF PUL INF 4711 4 MO 27 DAYS
BLW ♂ INT INF 5940 10 MO 8 DAYS
NO ISSUE
BORN JUNE 1, 1913
ALB. ♀ TAPEWORM 6901 9 MO. 20 DAYS
3RD LITTER
BORN MAY 31, 1913
BLW ♀ OCCLUSION OF STOMACH 6326 7 MO. 12 DAYS
BLW ♂ TAPEWORM 6155 6 MO 17 DAYS
BLW ♀ TAPEWORM 7997 1 YR. 2 MO 7 DAYS
BORN DEC 21, 1913
♂ CHR NEPH 8537 9 MO 27 DAYS
BLW JAP ♀ TAPEWORM NEMATODES 8692 10 MO 16 DAYS
BORN DEC 1, 1913
ALB ♂ UNK INF 8539 9 MO 17 DAYS
BLW ♀ TAPEWORM 7949 8 MO
BLW ♂ INT INF 8985 1 YR. 16 DAYS
BORN MCH. 1, 1914
ALB ♂ INT INF 8559 7 MO. 18 DAYS
BLW ♂ ASCENDING SUPPURATIVE PYELO NEPH 8554 7 MO 18 DAYS
BLW ♀ INT. INF 8655 8 MO. 1 DAY
BLW JAP ♂ INT. INF 8694 2 MO
2ND LITTER
BORN APL 30 '14
1ST LITTER
BORN JAN. 27 '14
BLW ♀ UNK INF. 8603 4 MO 28 DAYS
BLW ♀ INT. INF 8486 1 MO
NO ISSUE
NO ISSUE
NO ISSUE
AVERAGE AGE
PARENT GENERATION 1 YR. 9 MO
FIRST HYBRID GENERATION 11 MO 8 DAYS
SECOND HYBRID GENERATION 10 MO
THIRD HYBRID GENERATION 5 MO

mouse. Mated within her own strain or hybridized with other Mus musculus derivatives, she yielded prolific sturdy strains.

The parent male 6074, a Japanese Waltzer, mated within his own strain or hybridized with any other Japanese Waltzer strain, produced strains sturdy and prolific as any Japanese Waltzer stock. But these two when cross bred, viz., female 6573 and male 6074, produced a meager strain which died out in the third hybrid generation in spite of every effort to maintain it.

This pair had only 9 young, born in 3 litters; 2 in the first, 4 in the second, 3 in the third. Every possible mating was made of these 9 young, but only 6 second-generation mice were secured. Three of the matings were wholly without issue. In no case was there more than one litter born, and 3 was the largest number of young obtained in a litter, the other two yielding only 1 and 2 mice respectively.

The second generation hybrids in their turn, yielded only 6 third-generation mice. The mating of these third-generation mice gave no offspring whatever and the strain thus died out in the third generation.

The individuals of the first hybrid generation from this cross were slightly smaller than the best Mus musculus derivatives, but larger than Japanese Waltzers. The mice in the second and third generations, however, were considerably smaller than the first generation hybrids and less vigorous by every criterion, viz., scantier coats, feebler pigmentation, less active, less prolific, and shorter lived.

The average age of the parent mice was 1 year 9 months. The average age of the first generation hybrids was 11 months 8 days. The average age of the second hybrid generation was 10 months. The average age of the third generation was only 5 months.

This strain, no. 479, is typical of every strain ever secured in this laboratory in crosses between Mus musculus or its derivatives, and Japanese Waltzers.

The type of mouse derived from these crosses undoubtedly explains the paucity of "takes" which Little and Tyzzer secured after the first hybrid generation. An examination of their tables shows that in no case did they use any mice beyond the third hybrid generation and the diminution in their number of individuals from the first to the third generation is striking. It is evident that their results in these hybrid crosses were similar to those of this laboratory. It is to be expected that these feeble, short-lived strains would give a constantly diminishing number of "takes" as they give a constantly diminishing number and vigor of offspring. Therefore it is unnecessary to go far afield to find a type of inheritance to fit this number of "takes," particularly where such results conflict with the exact evidence of the inheritance behavior of spontaneous tumors. These authors were dealing with a feeble strain, which neither in offspring nor in tumor "takes" was vigorous like the parent strains. Whatever remains to be explained in their results would be furnished by the fact that in mass breeding the chance selections made for matings might pass by those mice which would have yielded progeny not immune to J. w. A.

To recapitulate then: where the hybrid strain is a feeble one like that resulting from the crossing of the Japanese Waltzer with Mus musculus and its derivatives, inbreeding lowers the progeny and the tumor production with more than normal rapidity, and speedily eliminates the strain.

There remains one point to be discussed in connection with the influence of inbreeding upon tumor production, viz., does inbreeding increase or does it decrease the ratio within a strain between the production of progeny and the incidence of tumor?

Lathrop and Loeb, in their paper quoted from above, state that in general the tumor rate is static within a strain, although in some few strains it shows a rise and in many cases it shows a fall.

Little and Tyzzer, on the contrary maintain that there is a steady decrease in the number of tumor "takes" of increasingly later generations, in their experiments, and deduce therefrom the application of a multiple-factor hypothesis for the inheritability of cancer. Their experiments, as stated above, were limited to three generations.

In considering this conflicting evidence, it must be remembered that Lathrop and Loeb are dealing with spontaneous tumors, while Little and Tyzzer are dealing with grafts. No further evidence of the intrinsic difference between spontaneous and grafted tumors is needed than Tyzzer's own results in later obtaining spontaneous tumors in individuals which had refused grafts (12).

Moreover, as stated above, Little and Tyzzer were obviously not demonstrating the inheritance behavior of "takes" of grafted tumor, but only the biological relation between tumor "takes" and a stock of mice of low grade metabolism and productivity. Their results were exactly what would be expected from such low grade, non-prolific stock.

A very large amount of evidence on the subject of the ratio between the production of tumor and of progeny has accumulated in this laboratory during the many years devoted to the study of spontaneous tumors. Obviously not all of this evidence can be brought within the confines of a single paper.

I have therefore gone over the strains already charted in Reports 5, 7 and 9 published from this laboratory, as these strains were selected with no reference whatever to the point here under discussion, and hence will yield typical and wholly unbiased evidence on the subject.

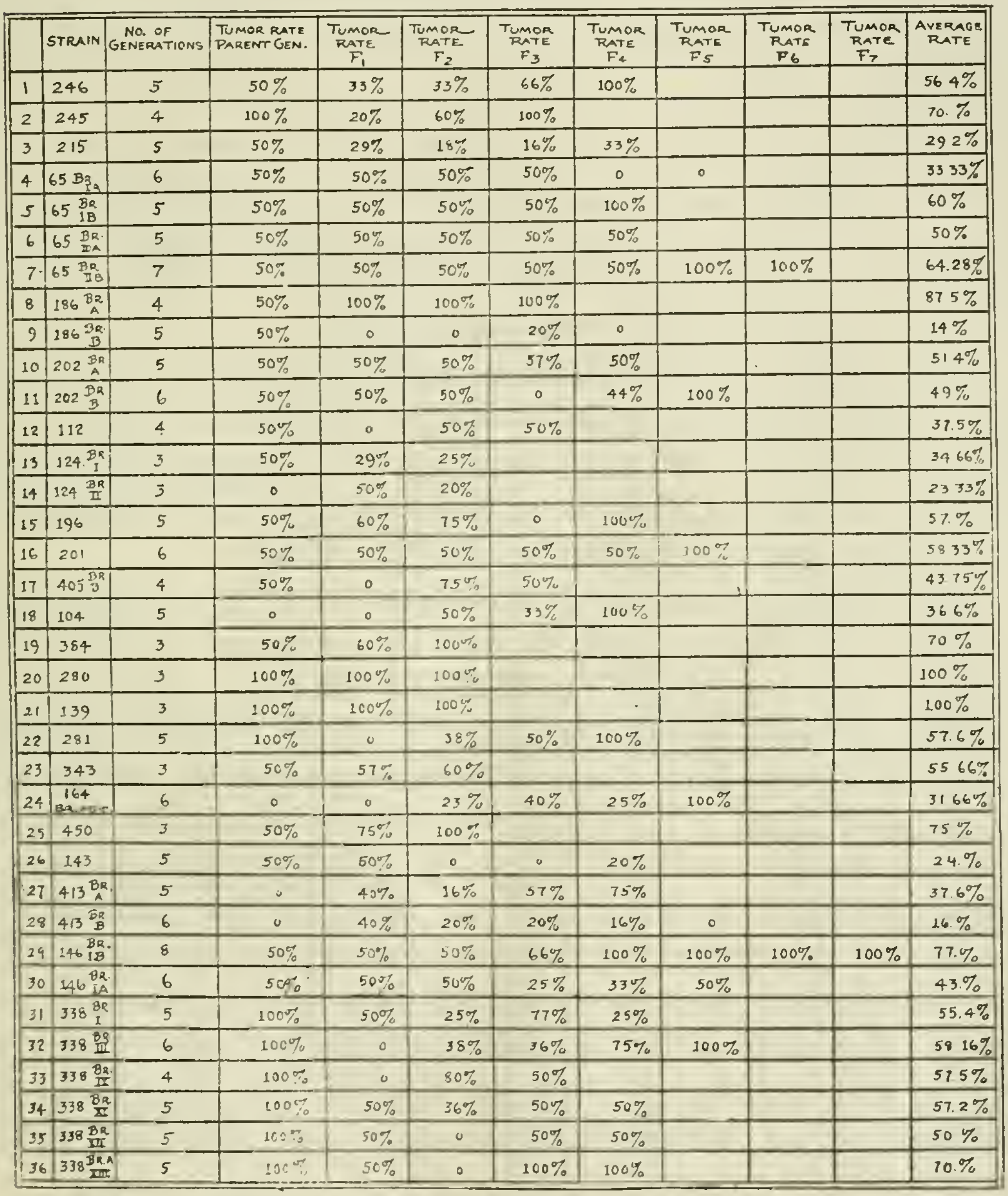
Chart 9 has been drawn up to show thirty-six of these strains which have already been published giving the number of the strain, the number of generations through which it was charted at the time of publication, the percentage of tumor production in the parent generation and in each succeeding filial generation, and the average rate of tumor production for the entire strain.

For example: Strain 246, charted through 5 generations, showed 50 per cent of tumor in the parent generation, 33 per cent in the first filial generation, 33 per cent in the second filial generation, 66 per cent in the third filial generation and 100 per cent in the fourth filial generation, or an average rate of 56.4 per cent for the entire 5 generations, etc.

Several points must be borne in mind in the study of this chart: (1) The matings made in these strains were all made *to test the Mendelian behavior of cancer;* not to find out how many tumors could be secured. (2) The matings therefore are of four kinds, double cancerous parentage; single cancerous parentage; parentage cancerous on one side, and heterozygous on the

CHART 9

RATE OF TUMOR PRODUCTION
IN EACH GENERATION OF VARIOUS STRAINS



	STRAIN	NO. OF GENERATIONS	TUMOR RATE PARENT GEN.	TUMOR RATE F_1	TUMOR RATE F_2	TUMOR RATE F_3	TUMOR RATE F_4	TUMOR RATE F_5	TUMOR RATE F_6	TUMOR RATE F_7	AVERAGE RATE
1	246	5	50%	33%	33%	66%	100%				56 4%
2	245	4	100%	20%	60%	100%					70. %
3	215	5	50%	29%	18%	16%	33%				29 2%
4	65 Br_{Ia}	6	50%	50%	50%	50%	0	0			33 33%
5	65 $^{Br}_{IB}$	5	50%	50%	50%	50%	100%				60%
6	65 $^{Br.}_{IIA}$	5	50%	50%	50%	50%	50%				50%
7	65 $^{Br}_{IIB}$	7	50%	50%	50%	50%	50%	100%	100%		64.28%
8	186 $^{Br}_{A}$	4	50%	100%	100%	100%					87 5%
9	186 $^{Br.}_{B}$	5	50%	0	0	20%	0				14%
10	202 $^{Br}_{A}$	5	50%	50%	50%	57%	50%				51 4%
11	202 $^{Br}_{B}$	6	50%	50%	50%	0	44%	100%			49%
12	112	4	50%	0	50%	50%					37.5%
13	124 $^{Br}_{I}$	3	50%	29%	25%						34 66%
14	124 $^{Br}_{II}$	3	0	50%	20%						23 33%
15	196	5	50%	60%	75%	0	100%				57. %
16	201	6	50%	50%	50%	50%	50%	100%			58 33%
17	405 $^{Br}_{B}$	4	50%	0	75%	50%					43 75%
18	104	5	0	0	50%	33%	100%				36 6%
19	384	3	50%	60%	100%						70%
20	280	3	100%	100%	100%						100%
21	139	3	100%	100%	100%						100%
22	281	5	100%	0	38%	50%	100%				57.6%
23	343	3	50%	57%	60%						55 66%
24	164 Br. [illegible]	6	0	0	23%	40%	25%	100%			31 66%
25	450	3	50%	75%	100%						75%
26	143	5	50%	60%	0	0	20%				24.%
27	413 $^{Br.}_{A}$	5	0	40%	16%	57%	75%				37.6%
28	413 $^{Br}_{B}$	6	0	40%	20%	20%	16%	0			16.%
29	146 $^{Br.}_{IB}$	8	50%	50%	50%	66%	100%	100%	100%	100%	77.%
30	146 $^{Br.}_{IA}$	6	50%	50%	50%	25%	33%	50%			43.%
31	338 $^{Br}_{I}$	5	100%	50%	25%	77%	25%				55.4%
32	338 $^{Br}_{III}$	6	100%	0	38%	36%	75%	100%			59 16%
33	338 $^{Br.}_{IX}$	4	100%	0	80%	50%					57 5%
34	338 $^{Br}_{XI}$	5	100%	50%	36%	50%	50%				57.2%
35	338 $^{Br}_{XII}$	5	100%	50%	0	50%	50%				50%
36	338 $^{Br.A}_{XIII}$	5	100%	50%	0	100%	100%				70.%

other; and double heterozygous parentage (the latter tested out to show that individuals may inherit and transmit cancer although they themselves do not exhibit it, just as is the case with albinism). (3) The parent generation, then, in each of these cases shows 100 per cent of cancer, or 50 per cent of cancer, or 0 per cent of cancer, according to what test was being made. (4) As has repeatedly been published from this laboratory, cancer rarely develops in mice under 6 months old, frequently it does not develop until the mouse is 2 or 3 years old; so that the cancer ratio (except in the 100 per cent cases) is nearly always lower actually than it is potentially (*i.e.*, if more mice lived to a greater age more would exhibit cancer). Infections creep in and sweep the mice off in numbers sufficient to lower the cancer rate very considerably.

Examination of chart 9 shows little falling off of the cancer rate except in sporadic cases where infections crept in. On the contrary, the later generations show almost uniformly a tumor percentage higher than those of the earlier generations or than the average percentage for the strain.

Note the percentages shown in chart 10 of the strains under consideration, 83 per cent showed the same or a higher percentage of tumor in generations later than the parent generation, 88 per cent of the strains showed the same or a higher tumor rate later than the first hybrid generation, 93 per cent showed the same or an increased tumor rate later than the second hybrid generation, 79 per cent showed the same or an increased tumor rate later than the third hybrid generation, 88 per cent of the strains showed the same or an increased tumor rate later than the fourth hybrid generation, 100 per cent showed the same or an increased tumor rate later than the fifth hybrid generation, 100 per cent of the strains showed the same or an increased tumor rate after the sixth hybrid generation.

Chart 11 compares each succeeding generation with its immediate predecessor, giving the percentage of strains showing a steadily increasing tumor rate in each succeeding generation. Note the percentages: 58 per cent of the strains showed a higher tumor rate in the first filial generation than in the parent generation. In 72 per cent of the strains the second filial generation showed a higher tumor rate than the first. In 71 per cent, the third hybrid generation exceeded the second in tumor production. In 75 per cent of the strains the fourth

CHART 10

PERCENTAGES OF STRAINS SHOWING

RISE OR SAME TUMOR RATE AS PARENT GENERATION	83%
RISE OR SAME TUMOR RATE AFTER F_1	88%
RISE OR SAME TUMOR RATE AFTER F_2	93%
RISE OR SAME TUMOR RATE AFTER F_3	79%
RISE OR SAME TUMOR RATE AFTER F_4	88%
RISE OR SAME TUMOR RATE AFTER F_5	100%
RISE OR SAME TUMOR RATE AFTER F_6	100%

CHART 11

PERCENTAGES OF STRAINS SHOWING

F_1 HIGHER THAN PARENT GENERATION	58%
F_2 HIGHER THAN F_1	72%
F_3 HIGHER THAN F_2	71%
F_4 HIGHER THAN F_3	75%
F_5 HIGHER THAN F_4	88%
F_6 HIGHER THAN F_5	100%

hybrid generation showed a higher tumor rate than the third. In 88 per cent of the strains the fifth hybrid generation showed a higher tumor rate than the fourth. In 100 per cent of the strains the sixth hybrid generation showed a higher tumor rate than the fifth.

We have here then conclusive evidence that as the cancer ancestry behind a generation broadens and deepens, whether by the method of inbreeding or by the method of hybridization, the individuals of that generation tend to run more and more to cancer production. Let us refer again to chart 9 and note how many of these hybrid strains became 100 per cent cancer strains, which originated from 50 per cent cancer parentage (*i.e.*, one cancerous with one non-cancerous parent or one cancerous with one heterozygous parent) or 0 per cent cancerous parentage (*i.e.*, two heterozygotes) strains 246, 65 branch I A, 65 branch II B, 186 branch A, 202 branch B, 196, 201, 104, 384, 164, branches A, B and C, 450, 146 Branch I B.

Note also the cases where 0 per cent cancer strains eventuated from 50 per cent cancer hybrid crosses. These were some of the strains where the purpose was to show that the segregating out of cancer and non-cancer make it possible *speedily to eliminate all cancer from the strain.*

It is obvious that where scientists oppose the theory of the inheritability of cancer and reject the mass of indisputable evidence, it is because they do not wish to accept a theory which they deem unfortunate for the human race; note Ewing's quotation from Le Doux-Le Bard and his personal subscription thereto: "In the interests of the public this doctrine" (i.e., heredity) "ought to be combatted" (13).

If scientists subscribing to this idea would concentrate their attention upon the following facts of heredity, they would find encouragement not hopelessness, in the unquestionable fact of the inheritability of cancer:

(1) In hybrid crosses cancer and non-cancer tendencies *segregate out* and are transmitted as such.

(2) All human matings are hybridizations.

(3) Cancer behaves as a recessive.

(4) It can therefore be wholly eliminated by persistently mating individuals of cancer ancestry with individuals with no cancer in their ancestry.

(5) This elimination is infinitely better than any therapeutic procedure.

(6) The cure of cancerous individuals during the reproductive period, makes it possible for them to transmit cancer to a greater number of progeny (14).

(7) The insistence that cancer is not hereditary, and the continued matings of two cancer-bearing individuals, results in an ever increasing amount of cancer in the human race. If persisted in long enough, such a method will eliminate all the perfectly non-cancerous families.

The progressive increase of cancer in later generations of hybrid crosses demonstrated above, must not be attributed to inbreeding, as exactly the same progressive increase of cancer follows where every mating is a hybridization, if the cancer tendency is bred in constantly in each succeeding generation. In other words, it is *what is put into a mating, not the method of putting it in, that determines which characters shall appear in the offspring.*

CONCLUSIONS

1. Inbreeding is demonstrated not to be an influence in the increase or the incidence of cancer, but merely a method of analyzing a strain.
2. This method of inbreeding is of necessity used by every student of heredity, as there is no test of heredity which does not involve inbreeding.
3. Strains consistently inbred may produce 100 per cent, or 50 per cent or 0 per cent of cancer according to *how much cancer is bred in*, not in accordance with the method used.
4. The real effect of inbreeding is to eliminate cancer by eliminating the strain. It is hybridization which increases cancer by increasing the output of cancer progeny.
5. The ratio of tumor "takes" in increasingly later generations from hybrid crosses of low grade productivity, proves

nothing with reference to the inheritability of cancer, but demonstrates the biological relation between race vigor and the number of tumor "takes."

6. As the cancer ancestry behind a generation broadens and deepens the individuals of that generation tend to run more and more to cancer production. This is equally true of inbred and of hybrid generations, since the amount of cancer which comes out in the progeny depends upon the amount which is put into the ancestry, whether the method is inbreeding or hybridization.

7. It is therefore possible wholly to eliminate cancer from the race by not putting it in through the ancestry; this is true both in inbreeding and in hybridization.

8. In demonstrating the inheritability of cancer and of other tumor types in mice we have demonstrated their inheritability also for man and for every other species in which they occur, since cancer and non-cancer tendencies which *segregate out* in mice must segregate out also in every other species in which they occur, and this is the test of heredity.

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PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH

TWELFTH ANNUAL MEETING

Held in Atlantic City, June 14, 1919

1. Report of the Council

The meeting of the Council was held at the Chalfonte Hotel, in Atlantic City, on the evening of Friday, June 13, 1919, i.e., the night preceding the day of the Twelfth Annual Meeting.

The following members were present: Dr. E. R. LeCount, president; Dr. William H. Woglom, secretary and treasurer; Dr. Harvey R. Gaylord, Dr. Francis C. Wood. Absent, Dr. H. Gideon Wells, Dr. James B. Murphy, and Dr. Robert B. Greenough.

The report of the treasurer included the sum of $125, $100 of which was given collectively by Dr. Harvey R. Gaylord, Dr. Benjamin F. Schreiner, Dr. B. T. Simpson, and Messrs. Millard C. Marsh and G. H. A. Clowes, and the secretary was requested to write to each of these gentlemen a note expressing the thanks of the Council for their generosity.

Means for increasing the circulation of the Journal were discussed and Dr. Gaylord suggested that Mr. Curtis E. Lakeman, secretary of the American Association for the Control of Cancer, be approached by Dr. Wood and asked to help in any way that he could to make the Journal known among members of the medical profession.

The names of three applicants came before the Council for election to the Association:

Dr. Ernest William Goodpasture, 7 Fremont Street, Reading, Massachusetts.

Dr. George H. Semken, 16 West 85th Street, New York.

Dr. Ross C. Whitman, University of Colorado, Boulder, Colorado.

Dr. Wood moved, and Dr. Gaylord seconded the motion, that these three applicants be elected to membership in the Association.

The resignation of the following member was accepted:

Dr. G. H. Mallett, 244 West 73d Street, New York.

Dr. Gaylord moved that Dr. Willy Meyer be elected councillor succeeding the retiring councillor. Dr. Wood seconded this motion.

The following officers were elected by the Council to serve for the ensuing year. Dr. H. Gideon Wells, president; Dr. Robert B. Greenough, vice-president; Dr. William H. Woglom, secretary and treasurer (re-elected).

The present Council, therefore, with the years of retirement, is as follows:

Dr. H. Gideon Wells, 1920
Dr. E. R. LeCount, 1921
Dr. F. C. Wood, 1922
Dr. James B. Murphy, 1923
Dr. William H. Woglom, 1924
Dr. Robert B. Greenough, 1925
Dr. Willy Meyer, 1926

The Council continued in office the present Editorial Board which is composed as follows:

Dr. William H. Woglom,
Columbia University
Dr. Frederick Prime,
Columbia University
Dr. Joseph C. Bloodgood,
Johns Hopkins University
Dr. Leo Loeb,
Washington University
Dr. Ernest E. Tyzzer,
Harvard University
Dr. H. Gideon Wells,
University of Chicago
Dr. James Ewing,
Cornell University

2. Lymphocytes and Cancer Immunity

Dr. Frederick Prime (New York):

SUMMARY

A series of experiments was undertaken in a strain of rats found to be resistant to repeated inoculations of the Flexner-Jobling rat carcinoma in 90 per cent of the animals, and in another strain of which but 15 per cent were resistant to the same tumor. The naturally immune rats were divided into two groups, one of which was given repeated exposures to *x*-ray, following the method advocated by Murphy, in amount sufficient to reduce greatly the lymphocytes in the circulating blood; the other group was kept as controls. Total white cell and differential blood counts were made before commencing the *x*-ray treatment, two days after the completion of the last exposure, and then at frequent intervals. Forty-eight hours after the completion of the last *x*-ray exposure all the groups of animals were inoculated with pieces of the Flexner-Jobling rat carcinoma, and the growths, if any, charted at weekly intervals thereafter. At the end of six weeks, out of forty-eight animals inoculated in each group those naturally immune animals whose lymphocytes had been reduced by *x*-ray treatment showed only 6.8 per cent of takes, whereas those which had not had any *x*-ray treatment showed 5 per cent of takes; a group of untreated controls, susceptible to the tumor, gave 90 per cent of takes. This experiment was repeated many times in both naturally immune rats and those proved immune through previous inoculations. In none of these was there any appreciable difference in the susceptibility of the animals to tumor inoculations, whether they had had the number of lymphocytes decreased by repeated exposures to *x*-ray or not. The same condition was found to be true also in rat sarcoma.

DISCUSSION

Dr. Francis C. Wood (New York): It seems to me that this paper rather effectively disposes of the postulated rôle of the lymphocyte as a carrier of immunity to cancer. I do not think it is possible to consider that the reduction of lymphocytes by the *x*-ray has any effect on the "taking" of tumors. What factors do play a part are unknown, but it seems to me that the lymphocyte as a carrier of cancer immunity disappears from now on.

Dr. W. C. MacCarty (Rochester, Minn.): In view of the fact that this paper is so contradictory to others, I would like to ask why this great variation has occurred.

Dr. Prime: I am afraid that the reasons for this variation have a great deal to do with the results that we get in all our work of this kind. Unfortunately a person working along one line in cancer research with too few controls gets one result and assumes a causal relation, and a second investigator who repeats his work on a larger scale gets a different result. It may be due to the tumor, or it may be due to the animal.

For example, the rats sold us by a certain dealer are almost or quite resistant to our rat tumors.

Dr. MacCarty: My reason for asking is this. I have a large series of cancer cases in human beings, and I am attempting to find out why some of them live so long. In patients where the glands are involved, earlier death might be expected. Yet some of them live eight or nine years, and I am at a loss to account for this.

3. The Relation of Pregnancy and Reproduction to Tumor Growth

Dr. Maud Slye (Chicago):

SUMMARY

In handling large numbers of mice with spontaneous tumors there is forced upon the observer from the very first the tremendous difference in the rate of tumor growth in non-reproducing and in reproducing females.

The same difference is noted in the non-reproductive and reproductive periods of the same female.

For this study thirty each of non-reproducing and of reproducing females with spontaneous tumors were selected. The tumors were all of the same type and of the same organ (with a few exceptions for purposes of comparison), viz., alveolar tubular carcinoma of the mammary gland.

Without exception the amount of tumor grown by a female while reproductive was much less than during her non-reproductive period.

Again, the amount of tumor grown by reproducing females was much less than that grown by non-reproducing females.

The normal course of these spontaneous tumors in mice that are not bred is very rapid, the mouse rarely living over six weeks and often less than a month after the appearance of her tumor. These tumors grow to a great size, frequently being as large as the body of the mouse.

However, when these tumorous mice are bred, the tumor scarcely grows at all during the reproductive period. The duration of the tumor is greatly prolonged, the mouse frequently living a year after the appearance of her tumor, during which time many of these mice bear from six to eight litters, aggregating from twenty to thirty-two young. When the mouse ceases reproducing, the tumor grows with tremendous rapidity and to great size, the female frequently living only six or eight days after the birth of the last litter. During this short period the tumor grows to many times its size at the date of the last litter.

In brief, during the six or eight days a tumorous mouse is non-reproductive, she grows much more tumor than during the eight months or a year that she is reproductive, the daily rate of tumor growth being far in excess of the daily rate during the reproductive period.

Two other factors must be taken into consideration, viz., (1) the age of the mouse, and (2) other complicating causes of death. Generally speaking, the younger mice show a higher daily rate of tumor growth than do the old mice.

Again, complicating factors, such as tapeworms or nematodes in the digestive tract, or any severe disease, greatly retard tumor growth.

But when these have been eliminated, two facts stand out with startling clarity and cannot be gainsaid: (1) Reproducing females grow much less tumor than do non-reproducing females of the same approximate age and general metabolic condition. (2) Reproducing females grow much less tumor while they are reproductive than while they are non-reproductive; in other words, when a mouse is producing embryos, she is not producing tumor in anything like the amount which she grows while non-reproductive. In striking contrast to these results is the relation between pregnancy and the infections common to mice.

If an infected mouse is bred, the infection is not held off for a year or more while she bears young; she is unable to produce any young at all and speedily dies of her infection. Or if a pregnant mouse contracts an infection, she rarely brings her young to birth.

The results of this study bring out with striking force the relation between tumor production and the production of young, showing them to be two related modes of growth.

DISCUSSION

Dr. Harvey R. Gaylord (Buffalo): I should like to ask Dr. Slye if she thinks it is possible to establish a strain of mice which will constantly produce a high percentage of tumors? I should like to inquire, also, into the frequency of tumors in virgin females as compared with animals which have been bred.

Dr. Slye: During the past ten years I have established hundreds of strains yielding a practically constant percentage of tumors. However, accidents which cause early death, epidemics of infection, lowering of the reproductive potency of a strain by insufficient diet or any other cause, or the running out of a strain by inbreeding, will of course lower the tumor percentage just as it will lower the reproductive percentage. I have shown before this Association strains showing 100 per cent of lung tumor or of mammary gland tumor, or 8 or 25 per cent of mammary gland tumor, or 60 per cent of liver tumor or ovarian tumor, etc.

The second question I am not prepared to answer at this time, as I have not yet compiled statistics on that point.

Dr. Gaylord: What I desire to determine is whether, in your opinion, a duly established strain, eliminating accidents, could be expected to continue to produce a definite percentage of tumors with such certainty that they could be used as the basis for experimentation. In other words, are the strains sufficiently constant in their percentage, after once established, to enable you to make deductions from specific experimental procedures?

Dr. Slye: I should not like to assume anything; in this work assumptions are dangerous. If you should take over a strain of my mice which was bearing 60 per cent of tumors in my laboratory, it might not go on producing 60 per cent in your laboratory.

Dr. Gaylord: If you have an established strain, I want to know about the stability.

Dr. Slye: There is no doubt about the stability of these strains, but they will run out, through inbreeding. As long as the strain persists, however, it will furnish tumors in a practically steady percentage, if it is correctly handled.

4. The Lymph-Nodes in Tumor-Bearing Mice

Dr. William H. Woglom and Dr. S. Itami (New York):

SUMMARY

This work is an extension of preliminary observations reported by one of us last year. At that time it was said that the lymph-nodes from rats with progressively growing or receding tumors exhibited no deviation from the normal, with the exception, perhaps, of some slight hyperplasia in the germinal centers.

The present series comprises a total of 213 animals—8 mice with spontaneous tumors, 129 mice with transplanted tumors, and 76 rats with transplanted tumors. The tumors were carcinoma, sarcoma, and carcinosarcoma.

It is not easy to establish a normal standard in these animals. Some nodes from supposedly healthy ones show a high degree of endothelial proliferation, others dilatation of the lymph-sinuses, still others a moderate hyperplasia of the germinal centers, and so on. On the whole, however, where a tumor has grown for five or six weeks an activity of the germinal centers in the lymph-node nearest the growth is sometimes found, and this activity is often somewhat more distinct than in mice without tumors.

The corresponding node on the opposite side is unaffected, whence it may be assumed that the activity is not due to a general systemic change. It is rather to be referred, perhaps, to some such local condition as the presence of a growing tumor, often slightly infected or containing necrotic material. This explanation seemed to us the more probable when we found, by injecting India ink, that the node studied in these experiments (the axillary node nearest the median line) is the one which drains the site at which the tumors were transplanted. Furthermore, nodes near receding tumors show a rather high degree of germinal activity. In any case the differences are slight, and it is not desired at this time to lay any particular emphasis upon them.

The number of nodes from spontaneous tumor mice is too small to serve as a basis for generalization; but it may be said tentatively that some of these nodes show a moderate degree of activity in the germinal centers and that others show little or none.

DISCUSSION

Dr. Wood: I think this illustrates very well what we know of human tumors of the breast, for example. We usually find such changes in human nodes more often in tumors which are either of the chronic mastitis type, where a great deal of absorption takes place from the ducts of material which is probably in many instances bacterially contaminated, or is irritating, as is shown by the cellular reactions which are produced around these ducts. Or we find it in carcinomata in which there is more or less necrosis of tumor cells.

Dr. E. R. Le Count (Chicago): What are the changes in the nodes? Are they in the germinal centers with overgrowth of lymphoid tissue, or are there changes in the sinuses as well?

Dr. Woglom: There are changes in the sinuses as well, but these occur also in mice that have no tumor. It is difficult to establish a normal standard. In mice apparently healthy there may be found an enormous dilatation of the lymph-channels, and half of the section or more may be made up of dilated channels. Sometimes these are filled with desquamated endothelium. Sections from other nodes will be composed almost entirely of lymphoid tissue, with the sinuses and germinal centers hardly visible; in still others the germinal centers are rather distinct. We have found no changes in the sinuses of tumor-bearing mice that could not be duplicated in nodes from mice without tumors.

Dr. Le Count: Is it chiefly, then, a growth of the lymphoid tissue?

Dr. Woglom: Yes, sir. The cells are larger and clearer, and there are perhaps a few more mitotic figures. But then there is more chance of encountering mitotic figures because the area of the germinal center is somewhat larger. The change, however, is probably of no importance so far as tumor immunity is concerned.

5. (*a*) CONCERNING THE DOSAGE OF RAYS

Dr. W. T. Bovie (Boston):

SUMMARY

If we look upon radiation as a flow of energy, we may indicate the dose by the total amount of energy absorbed, for it is a general law of photochemistry that the amount of photochemical change produced is, over a wide range of intensities, proportional to the total quantity of radiation absorbed. It easily follows that if the intensity of the radiation absorbed remains constant the amount of photochemical change is proportional to the duration of the exposure. But the intensity of the rays absorbed throughout an absorbing mass is not constant, for, due to spreading and absorption, the intensity decreases as the distance from the source increases, so that as the exposure proceeds equal increments of time do not result in equal increments of photochemical change.

It is impracticable to determine the relationship between the exposure and the amount of photochemical change by mathematical analysis. Using photographic paper, the author has mapped out equi-intensity surfaces about a linear source of radiation, such as a tube of radium emanation, both when the tube is embedded in a highly absorbing homogeneous medium, such as wax, and when only air is the absorbing medium. Intensity surfaces were also mapped out about tubes which were enclosed in the various applicators used in radiotherapy. From the shapes and dimensions of these surfaces, the influence of the volume of the radiated mass upon the amount of absorption was determined, and curves were exhibited showing the relation between the length of exposure and the total amount of photochemical change produced by the radiation.

5. (*b*) Concerning the Photo-Coagulation of Egg-White

Dr. W. T. Bovie:

SUMMARY

This is a preliminary report upon the influence of the hydrogen-ion concentration upon the coagulation by heat of a 10 per cent solution of egg-white which has been exposed to the ultra-violet radiation from a quartz mercury vapor arc. It was found that the greatest amount of coagulum is formed when the hydrogen-ion concentration of the solution is very close to its isoelectric point, and increasingly smaller amounts of coagulum are formed as the hydrogen-ion concentration is either increased or decreased. Similar non-radiated egg-white solutions showed no coagulum over the range of hydrogen-ion concentrations studied. The temperature of coagulation in these experiments was 18°C.

6. Incision of Tumors and Metastasis

Dr. F. C. Wood:

SUMMARY

In an experimental investigation of the effect of incising the Flexner-Jobling rat carcinoma upon the percentage of metastases, it was found that the incision of the tumor did not increase the percentage of metastases. It is, therefore, probable that the belief among surgeons that such incision necessarily hastens metastasis in human beings must undergo some revision.

DISCUSSION

Dr. MacCarty: I do not know how many times a year I have to decide the question of incision of tumors. I am very glad that these experiments have been done. I am glad also that Dr. Wood has been conservative, and speaks about *his* series of mice, because otherwise his conclusions might lead to indiscriminate practices. Whether or not tumors metastasize or grow more rapidly after incision does not make any difference in certain cases which I could mention. I am thinking of two or three instances illustrating this statement. One was a little child with a tumor on the sole of the foot. It had been treated by pastes, and a section had been removed by a surgeon and submitted to a reputable pathologist. He made the diagnosis of sarcoma and advised amputation. The case came to me for decision as to whether or not it was a sarcoma, and whether an amputation should be done. When I saw the section I decided that I would not make a diagnosis of sarcoma from the slide. The surgeon said "Now, what am I going to do?" and I said "I am going to incise the tumor, because if the child has a sarcoma it will never do the child any good to amputate. I shall not be satisfied until we examine the center of the tumor." So we did this, and I found a lipoma on the sole of the foot—quite a large lipoma, distinctly encaspulated, but possessing a zone of inflammatory tissue which had been produced by paste, and there was an area probably 1 cm. deep with growing fibroblasts as a part of the chronic irritation; the first pathologist had received a section of that area. Incision distinctly settled the question and saved the foot of the child. Whether or not we believe in the incision of tumors, it is sometimes absolutely necessary to cut into a tumor; but we do not advise the incision of tumors as a rule. Another case in point was a man with a lesion of some sort on his tibia. He likewise had had a section removed, and this was sent to laboratories and diagnosed by three pathologists. They were reputable men, good, honest, well trained pathologists, who were not intimately interested in the patient. The sections were brought to me. I always try to look at things from the clinical standpoint, and study not only the sections, but the patient. I refused again to make a diagnosis. The men who had made the diagnosis had legitimate reasons for doing so, according to many traditions in pathology, but they knew nothing about the case. I saw the patient, got his history, and reported to the surgeon that I refused to make a diagnosis of sarcoma; the patient might possibly have a syphilitic condition. They said, "What are we going to do?" I said, "We are going to operate on him and take another piece out." As a matter of fact, I did not find out from the slide that he had syphilis, but I could not be positive of sarcoma. It was before the day when the Wassermann reaction was much in favor. I had said to the man, "You have either syphilis or sarcoma. If you have sarcoma, you are not going to be cured. If you have syphilis, you have a very good chance." We cut into the lesion, and the surgeon said he would not amputate it. The patient had syphilis, but had failed to answer in the affirmative. That is another example where it is absolutely necessary to cut into a tumor, whether or not you believe incision is a good thing. Almost daily the orthopedic surgeon comes in and says, "I have a bone tumor which I want you to see." I see the patient and the x-ray pictures, but how can I tell from these what is in the tumor? It is only fair to the patient to cut in and find out what is there. So far as my experience is concerned, I have not seen any bad results caused indubitably by the incision of tumors. I recall very vividly a man with an enormous sarcoma on his neck which was incised, and a week or two later it was nearly twice the original size; but whether this was due to the incision I cannot say. As Dr. Wood has emphasized, I hope incision will not become a general custom; the general surgeon should not get the idea that he can cut into every tumor. I have not seen a breast tumor incised for many years. As long as we can go around a tumor we ought to excise, and never incise it, and certainly never massage it.

Dr. H. H. Janeway (New York): I wish to express my gratification to Dr. Wood for doing this experiment, and to say that his results confirm our impressions at the General Memorial Hospital, where we make biopsies as a routine. We have sent to us a great many inoperable tumors to be treated by radium, and we wish to have our diagnoses confirmed by microscopical sections. It has been our opinion that the incision is not accompanied by any diffusion of the growth. We have at the General Memorial Hospital a large number of cases strongly supporting this view, from which I will cite one example. A woman came to see us with a carcinoma of the tongue. A generous piece of the growth was removed two weeks before she applied to us for treatment. The section showed epidermoid carcinoma. This patient underwent a complete regression after one treatment by radium, and it is now two and a half years since this treatment was given, and she has developed no metastases to date. I give this as one instance of a large number of similar cases which impress us strongly that incision of a tumor for diagnosis is not harmful. I agree with Dr. MacCarty that much care in taking specimens should be exercised. In the majority of tumors of the breast we prefer not to make biopsies. In cases which are to be operated on there is no need of them.

Dr. Lockrey (Philadelphia): I had a tumor on my own tongue which was diagnosed as an epithelioma. There was a piece taken out, and it was found to be squamous-cell epithelioma. I had radium used, as well as *x*-rays, and an electric cautery. Three years have elapsed, and there has been no recurrence.

Dr. LeCount: It does seem as though it were necessary for someone to bring forward evidence to demonstrate that the opposite is true; in other words, the burden of the proof is with those who wish to demonstrate that incision is harmful.

7. Basal-Cell Epithelioma of the Rat

Dr. Dudley H. Morris (New York):

SUMMARY

This tumor was a very slowly growing variety, not continuously transplantable, but it grew for a long time when re-implanted in its original host. In other words, the tumor not only in its biological qualities, but also in its morphology, resembled very closely the basal-cell tumors of the skin in man.

8. Effect of Inoculation Technic upon Percentage of Tumor Takes

Dr. E. G. Cary (New York):

SUMMARY

The usual procedure is to wet the skin with 95 per cent alcohol and to insert a large caliber platinum needle carrying the graft under the skin of the abdomen, pushing it into the axilla where the tumor particle is deposited by a trochar. The method has been criticised as leading to bacterial infection or the tumor, but a series of experiments was tried where the skin was prepared in various ways, by epilation, shaving, and disinfection, especially with iodine. It was found that no greater percentage of takes could be obtained by such refinements, and that the tumors growing by the ordinary method of implantation are no more apt to contain bacteria than those implanted with the most painstaking surgical technic. It is therefore unnecessary to employ in tumor transplantation any especial means of disinfection of the skin of the animal to be grafted.

9. Arsphenamine and Tumor Growth

Dr. F. C. Wood:

SUMMARY

There is no reason to think that the administration of arsphenamine will stimulate tumor growth, and it is well known that neither it nor compounds of arsenic cause tumors to regress. It is always difficult to judge of the rate of tumor growth in man, even when untreated, and as there have been several instances where patients or their families felt that tumors were stimulated by the giving of arsphenamine, and had threatened legal proceedings, it seemed worth while to conduct a small series of experiments to note the effect of the administration of the drug. A series of mice was inoculated with a tumor, the growth rate of which was known, and the developing neoplasms were carefully measured at regular intervals. Not the slightest difference in growth rate could be detected by careful measurements between those animals receiving none of the drug and those which received varying dosages. It may be concluded, therefore, that arsphenamine neither stimulates nor inhibits tumor growth.

10. Attempt to Influence Tumor Growth

Dr. F. C. Wood:

SUMMARY

Several years ago Benedict stated that the administration of phlorhizin caused the disappearance of the Buffalo rat sarcoma, even when the tumors had grown to large size. His work was repeated by Wood and McLean with negative results. Benedict replied that the reason for this failure to confirm his results was that an insufficient quantity of phlorhizin had been given, and explained that he had used large rats and had periodically suspended the administration of the drug to enable the animals to recover from the toxic effects. The work was therefore repeated by Wood, following Benedict's technic, with negative results, but the findings were not regarded as of sufficient general importance to warrant publication until it was called to our attention that Ewing, in his recent book on neoplasms, has cited Benedict's work as valid. It seems desirable, therefore, to record the fact that these experiments were repeated with phlorhizin from three different makers, and on large animals, the doses being spaced as was done by Benedict. Not the slightest effect was noted on the Buffalo rat sarcoma. It is evident that Benedict's results were due entirely to spontaneous disappearance of the tumor and not to any therapeutic effect of the phlorhizin, thus confirming the published conclusions of Wood and McLean.

DISCUSSION

Dr. Bovie: This discrepancy in results reminds me of some blood counts. The clinician had drawn a very nice curve showing the results of radium treatments. It was a thin line, an average of several curves. I suggested that he represent the averages by a line wide enough to include a 50 per cent error in the data. This was some six months ago, and so far as I know the data have not been published. The width of the average curve drawn to include one-half of the data was so great that the curve was practically meaningless. A statement of only the averages of experimental data may be very misleading.

Dr. Wood: Dr. Prime and myself have spent many weary hours in attempting to compute the errors in lymphocyte counts. I had planned to use a similar wide strip showing the error inherent in the enumeration methods as given in Ross' error tables. We came to the conclusion that no two mice ever have the same blood count even approximately, and that there is no use in trying to make an average.

11. Pathological Changes Accompanying Injections of an Active Deposit of Radium Emanation

Mr. Halsey J. Bagg (New York):

SUMMARY

Following injections of an "active deposit" of radium emanation, by either the intravenous or subcutaneous method, there is a diffusion of the radio-active substance throughout the animal body, which subsequently results in pathological changes in the various organs. These changes were found to occur in the liver, lungs, kidneys, adrenals, spleen, bone marrow, brain, and the vascular system. The most interesting changes were those found in the liver, resulting from comparatively small doses of radium injected subcutaneously. A fatty degeneration, associated with many giant cells and hyperchromatic nuclei, was found in the liver for a comparatively long time after the treatment.

Following large doses of radium, congestion and hemorrhages were frequently found in practically all the organs, and in the severe, acute cases the animals died after showing symptoms of marked enteritis.

The most frequent pathological condition in the kidney was a granular degeneration and erosion of the renal cells. In the bone marrow there was a destruction of the cells, resulting in subsequent replacement by blood. Congestion of the spleen was the most constant feature following radium treatment, and in some cases this was associated with hemorrhages and the destruction of red blood cells.

The method of injection appears to determine, to a certain extent, the severity of reaction in certain organs. For example, following subcutaneous injections, there were no appreciable lung lesions, but with intravenous doses of about the same strength, proliferation and desquamation of the epithelial cells of the bronchi, marked edema, congestion, and hemorrhage were found to occur.

A similarity was noted in the tissue reactions when radium was injected intravenously or subcutaneously, and when it was applied externally.

Concerning the fate of radium after its injection into the animal organism, it was found that the liver, gastrointestinal tract, kidneys, lungs, and spleen received the greatest amount of radio-activity.

DISCUSSION

Dr. James Ewing (New York): Of course it is obvious that the object of this work is to extend the therapeutic action of radium from a local agent to a constitutional one, for we have to admit that up to date radium is not this sort of a therapeutic agent. I am very much interested in the results Dr. Bagg has obtained, especially in the ones which would lead to caution in the use of intravenous injections. In animals that are in a fair state of health sixty or seventy days after injection we find in the liver a very pronounced, and in my experience, specific lesion. Of course I use the word "specific" with every caution and reserve. I have never seen anything like it—huge hyperchromatic nuclei, extensive changes in the cytoplasm, and a lesion which in my experience is specific. The fact that relatively small amounts of the agent can produce a lesion which develops or persists after sixty or seventy days have elapsed would certainly suggest caution in employing this agent in human beings, because we do not know what is going to happen a year after the injection. One of the conclusions that I have drawn from this work is that one should count on a period of observation of not less than six or twelve months before estimating the effect of radium deposit on the patient. It is quite obvious from Dr. Bagg's work and from the work of others before him, that it is possible to obtain a constitutional effect, and a very pronounced effect, from injections of radium deposit. Just what it will lead to in the treatment of disease is hard to say. The lymphomatous group suggests itself as the first type of disease in which to use it. Back in 1910 in Paris, I was told by a French observer that he had seen the disappearance of a lymphosarcoma in a child after the injection of radium salts. On the basis of this work Dr. Janeway has already carried out some clinical observations on the effect of radium deposit injected intravenously.

Dr. MacCarthy: I should like to ask whether the clinicians who have been using radium have noted any degenerative changes in the rest of the organs, and whether the action of radium is transferred to other parts of the body from a local point.

Dr. LeCount: I should like to ask if the spleen is enlarged, and also whether these rather interesting changes in the liver can be explained.

Dr. Bagg: In regard to the first question, I do not know whether any scientific work has been done. I believe the General Memorial Hospital is one of the first to employ the method. Perhaps Dr. Janeway can answer your question.

Dr. Janeway: Our experience in the injections of active deposits has been limited to two cases. Unfortunately peculiar circumstances made it impossible for us to carry out the examinations necessary to answer Dr. MacCarty's question. The most we were permitted to do was to make blood counts at intervals on these patients. I wish to emphasize again the need for caution in drawing conclusions of which Dr. Ewing has spoken. The first patient received an injection in October. He left the hospital and I traced him with considerable difficulty. The second patient left the hospital after the injection, and we were able to trace her also with considerable difficulty. I had a second blood count made in her case at the hospital about two weeks ago. I do not think I can make any statement regarding the manifestation of the toxic changes such as Dr. Bagg speaks of from the result of the clinical examination of these patients. There is no doubt that the injection does produce very severe toxic effects. The second patient I treated received rather a large dose. The reaction after it demonstrated what constituted a maximum or even an over-dose. She was prostrated for at least three weeks' time, and for five days after the injection she vomited continuously. However, as far as can be told to-day, the end result is encouraging. She received the injection in February. At that time she had a white blood count of 30,000, with 100 per cent of lymphocytes. When I last saw her the blood count was 10,000, with practically a normal distribution in the differential count. She recovered after a month's time from her period of prostration but, as Dr. Ewing has said, this does not mean that there may not still be severe changes in the internal organs. However, she now feels better than for a long time before receiving treatment.

Dr. Bagg: In regard to the question of tissue changes, I do not think we can formulate any definite theory, but it does seem possible that this very active substance, coming in contact with the cells, may change the permeability of their membranes. It looks as though a considerable amount of water had been taken up by the cells, and as though following that phenomenon there had occurred a granular and fatty degeneration. It is quite possible that this change may have resulted in the metabolism of the cells being disturbed, and that being the case, a subsequent degeneration might be expected.

Dr. Le Count: Is the spleen large?

Dr. Bagg: Usually it is somewhat reduced in size. I think that there has been a certain amount of clinical observation showing like results following similar methods.

12. (*a*) FLUCTUATIONS IN INDUCED IMMUNITY

(*b*) FLUCTUATIONS IN CONCOMITANT IMMUNITY

Dr. F. D. Bullock and Dr. G. L. Rohdenburg (New York): Read by Dr. Rohdenburg.

SUMMARY

(*a*) The authors call attention to the fluctuations in the percentage of induced immunity observed in various experiments. The importance of the recognition of these variations is emphasized, and evidence is produced which apparently proves that they are due to the neglect to use pure bred animals as hosts. When pure strains are employed as recipients of the tumors, the variations are negligible.

(*b*) Attention is called to the fact that the behavior of a tumor as an immunizing agent is not constant. From the various experiments recorded it appears that the variations are due to changes in the tumor and not to changes in the host. It was shown in the previous paper that analogous variations in induced immunity were due to variations in the strain of animals used.

DISCUSSION

Dr. Woglom: There is one aspect of Dr. Rohdenburg's work which might have been attacked if we had not been careful to control it. I refer to the personal equation in interpreting the results, a factor which always has to be taken into account. I took his charts and, without seeing his curves, constructed curves of my own from them; it was remarkable to see how the two series coincided.

Dr. Wood: Another point that leads to impartiality in the Crocker Laboratory is the fact that the tumors are all charted by a laboratory helper who knows nothing of the experiment. No investigator charts the tumors with which he is working, so that the personal element in determining the size of the tumor is eliminated.

Dr. Ewing: I should like to inquire what the significance of all this is regarding the nature of what you call immunity.

Dr. Gaylord: It seems quite logical that Drs. Bullock and Rohdenburg should have finally covered this most interesting phase of cancer research by careful study. The conflicting reports of various experiments in attempting to repeat work of a positive character previously published, has convinced all of us in the past that very striking fluctuations in the conditions surrounding these experiments really existed. I recall that the Buffalo rat sarcoma at one time gave alternate high and low percentages of successive inoculations in each alternate generation over a considerable period, and in its relation to immunity von Dungern has already emphasized such fluctuations. In the early days of experimental cancer research, positive findings were controverted by negative results in the hands of other experimenters, and in many cases these questions became controversial. This is an extremely interesting and satisfactory paper and should definitely clear this field. It will serve also to re-establish much positive work which has frequently been apparently disproved, and it will greatly strengthen the foundation of experimental research in tumors and immunity in animals.

Dr. Isaac Levin (New York): The real difficulty in all our research in cancer immunity lies in the fact that the cancer cell has peculiar characteristics, and does not behave in an identical manner at different times. This is true in animal as well as in human cancer. A tumor may lie quiescent for several years and then, without the action of any extrinsic agent, become more or less malignant. It is quite possible that the different results often obtained by several investigators with the same tumor and the same method of immunization, may be due to the variability of the tumor employed.

Dr. Le Count: Has anyone any observations bearing on fluctuations and their behavior in a standard tumor stock which has been used for many years?

Dr. Levin: I have been working with the Flexner-Jobling tumor for several years, and I can testify that it has behaved differently as regards its transmissibility during that time.

Dr. Le Count: Is this the experience of others working with the same stock?

Dr. Gaylord: There are many instances of that kind. In our first experiments in immunity, a controversy arose in regard to our results; and though we tried several times to repeat them, we could not. After two or three years we were able to do so.

Dr. Rohdenburg: In answer to Dr. Ewing's question, I shall have to admit that these experiments do not show very much. Originally we were trying to solve a problem which came up several years ago: the problem of what particular factors are at work in the production of immunity in animals. Varying results were being reported, and the question arose, who was right. As it turned out, any of them may, perhaps, be right for a given tumor at a given time. I think Dr. Gaylord is correct in saying that, as a result of this demonstration, we may be able to correlate many things that at present are rather at variance.

13. MALIGNANCY OF THE CROWN GALL AND ITS ANALOGY TO ANIMAL CANCER

Drs. Isaac Levin and Michael Levine (New York). Read by Dr. Levin.

SUMMARY

Previous investigations of the writers have indicated that the crown gall presents an ideal material for the cellular study of the cancer problem. It appeared imperative, therefore, to estimate the true relationship between the crown gall and animal cancer, and this was the object of the present investigation. A large number and a great variety of plants were inoculated by Dr. Smith's method. The research shows that certain of these plant-tumors behave, both morphologically and biologically, as benign growths; while others, inoculated in the same manner, appear to be true malignant tumors. Certain phases of the development of these tumors resemble granulomata as near as an organism void of blood or lymph circulation may do so. In accordance with the findings of Dr. Smith a number of crown galls were obtained containing leafy shoots.

The microscopical study of the material revealed conditions which differ materially from the conditions obtained in animal cancer. Usually the entire gall presents a uniform morphological appearance of small, young, undifferentiated cells. In other galls the central growing part presents the usual appearance of a crown gall, while the periphery shows the development of adult differentiated tissue (parenchyma), rudimentary organs (conducting system), or even a whole rudimentary organism (leafy shoot).

The conclusion to be derived from this study is that a rapidly developing simple crown gall presents considerable analogy to animal cancer, and offers an ideal material for the cellular study of the latter condition. On the other hand, the structure of the growing central part of the crown gall does not change with the structure of tissue inoculated. It represents, therefore, only one form in the large group of pathological growths designated under the name of cancer. Until it can be demonstrated that the same microorganism may produce a crown gall with an entirely different morphological structure derived from a selected tissue it cannot be asserted on the basis of this study that one parasite must be the cause of all human cancer.

DISCUSSION

Dr. LeCount: I am sure that you all remember the interest aroused several years ago by the work of Dr. Smith; and perhaps you have been often asked, as I have been, whether proof had not at last been brought forward that cancer is a parasitic disease. I think this paper should be fully discussed.

Dr. Gaylord: I want to ask Dr. Levin if he does not consider that the question of tissue differentiation in crown gall is not well covered by the marked differentiation found in the transplanted osteochondrosarcoma of chickens. This tumor, one of Rous's caused by a filterable agent, is a tumor composed of cartilage and bone, portions of which cease growing and lose their neoplastic characteristics, leaving spheres of fully developed bone containing red bone marrow. Dr. Levin says that the peripheral cells at the margins of one of the crown galls, of which he shows us a lantern slide, have lost the tumor character and present the appearance of normal plant cells. I should like to ask whether he considers that in these cells the process of crown gall has terminated, leaving differentiated cells behind. If he makes that interpretation, then the conditions are exactly analogous to those in the osteochondrosarcoma of chickens, and his contention that these conditions in crown gall are different from anything found in animal pathology is not well grounded. The question of the analogy of crown gall to tumors in animals was emphasized by Jensen in 1910, when, at the meeting of the International Cancer Congress in Paris, he discussed these plant tumors, in showing some very interesting specimens of crown galls implanted from white beets into red ones. He stated that the analogy between the plant tumors and those of mice, with regard to transplantation, is extremely close; and after citing many points of similarity, said, furthermore, that the crown gall is, in his opinion, a true neoplasm because no etiological organism had ever been demonstrated. Curiously enough, at that time Dr. Smith had already demonstrated his *Bacillus tumorfaciens.* I should like to ask Dr. Levin if, in his opinion, there is any question of *B. tumorfaciens* being the sole cause of crown gall. Dr. Smith's interpretation of the significance of crown gall is just the reverse of Jensen's; Jensen regarded crown gall as a neoplasm because no organism had then been found in it, while Dr. Smith maintains that because this plant tumor is caused by a bacillus, its etiology affords strong evidence of the probable parasitic nature of neoplasms in animals and man. I have always felt that there would have to be some discount of any sweeping conclusions of this kind; the great variety of tumor formation in man, precludes in itself the possibility of a single causative agent.

Dr. Georgine Luden (Rochester, Minn.): I want to ask Dr. Levin whether he has tried to reproduce some experiments which were done last year or the beginning of the previous year by Dr. Smith, as a result of which he rather discounted the significance of his own bacillus, because he was able to get the same kind of tumor in plants by using dilute alkali, dilute acid, acetone, and various kinds of oils. He obtained the same results merely by exposing his plants to chemical vapors, and came to the conclusion that, as he was able to produce these same types of reaction by chemicals, the bacillus is not at the bottom of the proliferation, but that this is due to a chemical reaction in the plant, the chemicals contained in the bacillus, or in the plant itself being responsible for the formation of the tumors.

Dr. MacCarty: For a number of years I have been very much interested in Dr. Smith's work, both in his own laboratory and in my own, and within the last two months I have spent some time in his laboratory going over his material, so I know something about his work and the historical development of it. I think our speaker has been a little too rigid in his criticism; in my own opinion this work has been one of the most important pieces of biological research of the last twenty years, even though Dr. Smith may have made some mistakes in applying his results to animal pathology. If such occur, they do not seem to me to be very serious. One illustration which Dr. Levin uses I should like to question; his statement, namely, that tuberculosis in a node may become either Hodgkin's disease or lymphosarcoma. I should like to know if there is any scientific evidence that a tuberculous node can ever become either lymphosarcoma or Hodgkin's disease. In regard to differentiation in the cells of human neoplasms, I know of no case where cells have been completely differentiated. If they had been, they would have produced an accessory tissue or organ, not a tumor. In these tumors of Dr. Smith's, differentiation is incomplete, as it is in human neoplasms. Those tissues of the human body which are most highly differentiated in malignant neoplastic conditions are the cells of rectal carcinoma and of squamous-cell epitheliomata. But I have never seen any malignant neoplastic cell that could be called completely differentiated, after examination with a high power lens. The nucleus is not differentiated. I believe pathologists have been a little dogmatic in putting granulomata into one group and new growths in another; it seems to me that we are dealing with the same biological process of defensive reaction to environment, and that these two conditions are but phases in the biological reaction. Environment certainly varies in these cells. In neoplasms the cells tend to differentiate if the environment is favorable, but, as I said, I have never seen one completely differentiated. Instead of attempting to separate regeneration and neoplasia sharply from one another, we should take into consideration the question of texto- and cyto-differentiation and regeneration, which are most important factors both in regeneration and in neoplasia. I do want to emphasize my belief that when the essayist criticizes Dr. Smith's work and conclusions, he should consider the evolution of his studies. I have spent hours trying to convince Dr. Smith that these crown galls are not due to an organism. As a matter of fact, he is now producing neoplastic conditions by purely physical means; he can initiate them in certain begonias by merely transplanting from one pot to another, in other words, by an interference with constitutional conditions plus a local destruction. I am looking at these things from the purely biological standpoint, in dealing with the regeneration of cells. The nearer we come to looking at the question of neoplasia from the standpoint of regeneration, the nearer we shall come to the real biological facts relative to neoplasia.

Dr. Woglom: I should like to ask if these so-called embryomata arise only at the site of accessory buds; if so, may their importance not have been overestimated, since no unusual stimulus is required to complete the development of accessory buds? Again, may I ask whether the so called embryomata contain representatives of root, stem, and leaf? In order for these lesions to merit the term "embryomata," should not some of them at least have all three of these parts?

Dr. LeCount: It is very interesting to have applications made to animal pathology by a botanist, and to have similar experiments made by a worker in animal pathology, because only in this way are we going to ascertain the truth. In the lantern slide where there was crown gall in the center and a scar at the periphery, are the cells about the periphery crown gall cells? In regard to the osteosarcoma referred to by Dr. Gaylord, I once studied a malignant tumor arising in the upper end of the tibia[1] in which the tumor cells did nothing but make bone. They did not make cartilage at all. In this the growth differed from all the osteosarcomas I have seen. There were metastatic growths in the lungs, and upon arriving at the pleura the tumor cells had made bone there, which finally reached such a stage of maturity that no one could doubt the differentiation. One could not have distinguished that bone from bone altered a little by disease, or even from normal bone, because it had differentiated into complete Haversian systems, with lamellæ, bone marrow, depots of red blood cell genesis, and fat.

Dr. Levin: I fully expected that the discussion of my presentation would embrace the whole field of pathology and biology. Dr. Gaylord apparently still has some sympathies left for the parasitic theory of cancer. Chondroosteosarcomata and osteomata are tumors which consist of bone-forming cells, and are not comparable to either embryomata in man or to the crown gall, which forms leafy shoots. The latter are complete rudimentary organisms and are not comparable to anything observed in animal tumors. Now to take up the statement of Dr. MacCarty that I am criticising Dr. Smith's work, or undervaluing it; I am indeed far from it. I think he has done a wonderful piece of biological research, and I believe that the crown gall is the most ideal material for the study of biological phenomena of cell proliferation. I have no argument with the investigations of Dr. Smith, only with some of his conclusions. When an investigator in botany makes sweeping deductions in the field of human cancer, the latter urgently need revision. Our thesis is the "*Malignancy* of the crown gall and its relation to animal cancer," not the general investigation of crown gall. In answer to Dr. Luden: we carried out experiments with chemicals also, but what we obtained was not a true crown gall, and surely not a malignant tumor. All irritants will readily produce a wart-like structure, a benign growth, on a plant. Our investigations, which will be reported later, seem also to indicate that microorganisms other than the *B. tumorfaciens* may produce conditions analogous to a true crown gall. Dr. MacCarty doubts whether tuberculous nodes may change into Hodgkin's disease or into a lymphosarcoma; yet there is very authoritative opinion which claims that Hodgkin's disease is always due to the tubercle bacillus. But one thing is absolutely certain, and that is that Hodgkin's disease and lymphosarcoma always develop on the basis of a tuberculous or other infectious granuloma. I do not believe that Dr. MacCarty quite followed me in my remarks on cell differentiation. I maintain that tumors in the animal organism consist of undifferentiated tissue. I stated that crown gall is also an undifferentiated tissue, and thus far is analogous to animal tumors, but just when it ceases to be undifferentiated and develops into differentiated tissue with specialized function, then its similarity to animal tumors ceases. Dr. Woglom is correct; an embryoma usually occurs where accessory buds take place, and the buds may form as easily without the tumor. The leafy shoots always develop from the ordinary crown gall cells. In answer to Dr. LeCount's remarks, I can say that the adult differentiated stem cells observed at the periphery of the crown gall are newly formed from the crown gall cells. Again it must be stated that the leafy shoots developing at the periphery of the crown gall are not analogous to osteomata any more than to embryomata. Dr. Smith has made the mistake of overvaluing something, because he has not clearly understood the significance of human embryoma, of teratoma, and the subsequent development of a malignant tumor within the latter tumors. The leafy shoots or other specialized tissue develop subsequently to and upon a presumably malignant tumor, and therein lies the main difference between the animal and plant tumors.

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[1] Johns Hopkins Hospital Bulletin, 1909, xx, 361–370.

Dr. LeCount: If you had seen the peripheral tissue alone would you have known that it was crown gall?

Dr. Levin: No.

Dr. MacCarty: In regard to calling these embryomata and teratomata, I might state that when Dr. Smith had made his experiments he sent his results to me, and I went to Washington to keep him from calling them embryoma and teratoma. I am not in favor of calling such neoplasmata, either in plants or animals, by such names.

Dr. Gaylord: I think Dr. Levin misunderstood me entirely. I did not mean to infer that he was criticizing Dr. Smith. I have never sympathized with the idea that crown galls produce teratoid tumors. I have much the same opinion that Dr. Levin has. In regard to the remarks that have been made about inducing cell proliferation in plants with chemical and other means, that is not remarkable. The astonishing thing was the discovery that a bacterium produced a pseudo-tumor. I think that no one has any idea today that one organism is the cause of cancer, no matter to what extent they may believe that certain tumors are parasitic. But I think that everyone can see that cancer is a great group of diseases. I should take issue with Dr. Levin's remark about the origin of the osteochondrosarcoma of chickens. These tumors arise from undifferentiated connective tissue, the same undifferentiated tissue from which Rous has been able with two other viruses, one derived from a spindle-cell sarcoma and the other from a spindle-cell sarcoma which developed extensive clefts lined with endothelium, to produce tumors true to these types by injecting with these viruses. In this instance it appears that the virus of osteochondrosarcoma acts in such a way upon these cells as to cause them to form cartilage and bone.

Dr. Levin: Dr. Gaylord started by saying that he saw differentiation of tissues in a malignant tumor, and then changed it to a "somewhat" malignant tumor. No truly malignant tumor will behave in this manner. Moreover, as stated above, a bacterium easily incites cell proliferation in a plant, since this is the only way in which a plant may react to irritation. The *Plasmodiophora brassicae* causes a proliferation not only in the cells which contain the parasite, but also in the free cells in the neighborhood.

Dr. LeCount: Your Chairman feels that there is no disagreement needing further discussion.

14. (*a*) Tissue Transplantation and the Lymphocytic Reaction

Dr. Leo Loeb (St. Louis):

SUMMARY

In the case of homoiotransplantation of various tissues a lymphocytic reaction appears around the transplant, which is absent in the case of autotransplantation.

This reaction is a graded one, and indicates the relationship between donor and host. In case of transplantation into near relatives (from brother to brother) the reaction appears later than in the case of transplantation into individuals who are not related to each other. The degree of strangeness of the substances produced by different members of the same species varies in accordance with the degree of relationship between host and donor, and the lymphocytes sense these differences. The reaction of lymphocytes which is elicited through homoio- or syngenesioplastic transplantation is a primary reaction of the lymphocytes and not a reaction caused by immune substances.

The fibroblasts and capillary vessels are also specifically influenced by syngenesiotoxins and homoiotoxins. In the case of homoiotransplantation fibroblasts are attracted strongly by the transplant, while capillaries are attracted only weakly. The reverse condition holds good in autotransplantation. In syngenesiotransplantation the conditions are somewhat intermediate, but on the whole, especially so far as practical results are concerned, syngenesiotransplants approach more closely homoio- than auto-transplants. The reactions called forth through immunization against homoio-tumors resemble closely those found in the case of homoiotransplantation of normal tissues.

(*b*) THE INVASION OF BLOOD VESSELS BY A NORMAL TISSUE TRANSPLANT

Dr. Loeb:

SUMMARY

Regenerating tissue may show many characteristics of cancer tissue. We have recently had an opportunity to observe even the penetration of newly formed capillaries by regenerating strands of thyroid tissue. From here the invading tissue was carried to arteries, and within the lumen of the latter relatively large quantities of this tissue became fixed to the wall of the vessel and sheathed in by endothelium. The invasion of capillaries was not confined to one place, but occurred in several vessels simultaneously. The invading tissue not only remained alive in the vessel wall, but continued to proliferate mitotically.

This observation was made in a case of autotransplantation, seven days following the implantation.

While regenerating tissue may in certain important aspects closely resemble cancer tissue, still it would be false to conclude that cancer tissue is merely normal regenerating tissue which continued to proliferate because it did not make proper connections with its own kind of tissue.

LYMPHOCYTES AND CANCER IMMUNITY

FREDERICK PRIME

From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director

Received for publication June 16, 1919

Since, following the suggestions of Da Fano and others, Murphy (1) and later Murphy and Morton (2) have laid great stress upon the rôle played by the lymphocyte in the production of immunity, much work has been done with widely varying results. Sittenfield (3), for example, found that neither increase nor reduction of the lymphoid elements in the blood had any influence upon resistance or susceptibility to tumor growth, when the Flexner-Jobling rat carcinoma was used. With such differences in the results of published experiments it seemed advisable to continue investigations of this problem of tumor biology in order to correlate, if possible, the work already done. This experiment is one of a series undertaken with this end in view.

Recently the Crocker Fund obtained a strain of rats which was found to be resistant in about 90 per cent to repeated inoculations of the Flexner-Jobling rat carcinoma, the inoculated tumors receding after about fourteen days. Another available strain of rats, on the other hand, showed but about 15 per cent resistance to the same tumor. These naturally resistant rats, together with others of the same strain already proved immune to the Flexner-Jobling rat carcinoma by previous inoculations, were divided into two groups, one of which was given sufficient repeated exposures of *x*-ray greatly to reduce the lymphocytes in the circulating blood, while the other was kept as controls. Both total white and differential blood counts were made before commencing the *x*-ray treatment, two days after the completion of the last exposure, and subsequently at frequent intervals. In all cases the blood was taken from the animals under similar conditions. During the experiment all the animals remained under the same conditions as regards both feeding and treatment.

For five consecutive days the rats were given exposures of unfiltered x-ray from a Coolidge tube run from a Kelley-Koett rectifying transformer. The distance from the anticathode to the back of the animals was 30 cm., spark gap 3.5 inches between points, milliamperes 10, exposure four minutes, a total of 10 Holzknecht units being given. The pastilles were checked on

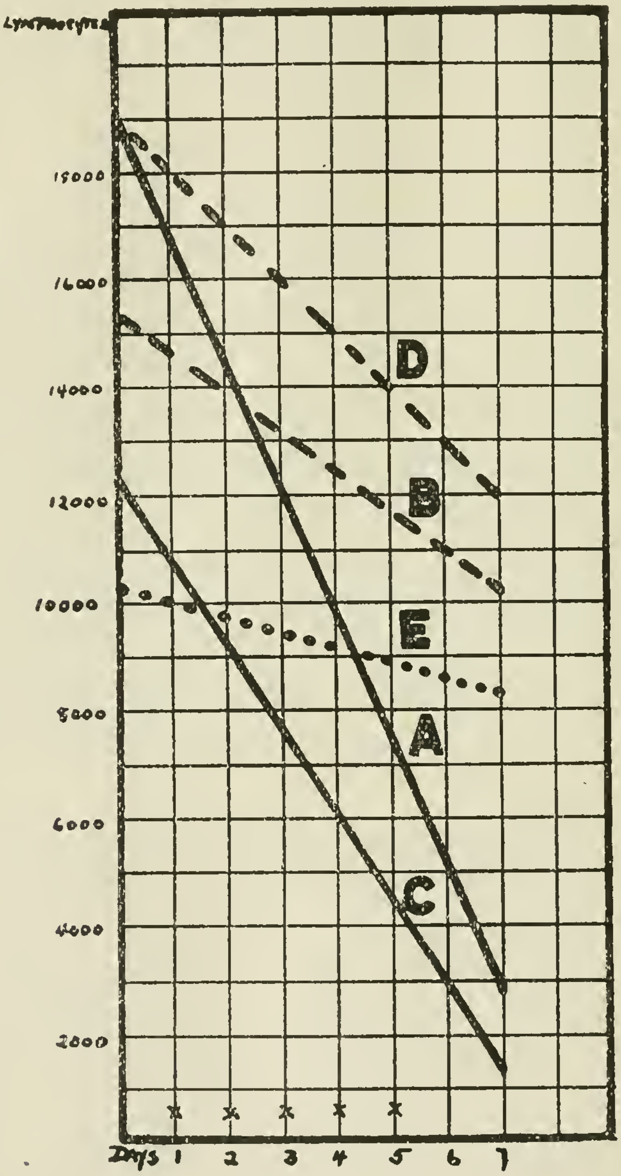

FIG. 1

both a Holzknecht and a Cox radiometer, the readings being very consistent. This is a smaller dose than that given by Murphy, but we found that when we used a total of 30 Holzknecht units, as he reports, our animals did not live long enough to complete our experiments. Text figure 1 shows the composite blood counts before, and forty-eight hours after the completion of the *x*-ray treatments. The blood counts of the control groups for the same periods also are shown on the same chart. Each line represents the composite lymphocyte count per cubic millimeter of blood of each group of rats.

Group A. Forty-eight resistant rats given *x*-ray.

Group B. Forty-eight resistant rats kept as controls.

Group C. Twenty-four immune rats, having had previous inoculations of Flexner-Jobling rat carcinoma; given *x*-ray.

Group D. Twenty-four immune rats, having had previous inoculations of Flexner-Jobling rat carcinoma, kept as controls.

Group E. Forty-eight non-resistant rats kept as controls for whole experiment.

TABLE 1

$\frac{\text{F R C}}{\text{66 C}}$	NUMBER OF RATS	POSITIVE	NEGATIVE	PERCENTAGE OF TAKES	PERCENTAGE NEGATIVE	EXPLANATION
Group A	44	3	41	6.8	93.2	Resistant stock; no previous inoculation; given reducing exposures of *x*-ray
Group B	40	2	38	5.0	95.0	Resistant stock; no previous inoculation; no *x*-ray
Group C	19	2	17	10.5	89.5	Resistant stock; previously inoculated unsuccessfully with F. J. R. C.; given reducing exposures of *x*-ray
Group D	21	1	20	4.8	95.2	Resistant stock; previously inoculated unsuccessfully with F. J. R. C.; no *x*-ray
Group E	40	36	4	90.0	10.0	Ordinary stock. Control for experiments

Forty-eight hours after the completion of the last *x*-ray exposure all the groups of animals were inoculated with pieces of Flexner-Jobling rat carcinoma, 0.003 gram of tissue being inserted subcutaneously through a hollow needle; and the growths, if any, were charted at weekly intervals thereafter. The number and the percentage of takes in the animals surviving at the end of the sixth week are shown in table 1.

Group E, which was the control for the adaptability of the tumor for the whole experiment, showed 90 per cent of takes after six weeks, all of the tumors being of large size. There was practically no difference in the inoculability rate of the tumors in the immune animals, irrespective of whether they had been given *x*-ray treatment or not. At the end of the sixth week, as the chart shows, most of the tumors had disappeared in the radiated as well as in the non-radiated groups. The few positive takes were very slow in appearing and made poor progress in comparison with the normal growth rate of the Flexner-Jobling rat carcinoma as shown in group E. In text figure 2 the actual number of takes for the sixth week is shown graphically.

The composite lymphocyte curve of groups A and C, which were given *x*-ray treatment, shows (see text figure 3) an enormous reduction in the number of circulating lymphocytes compared with the control groups B, D, and E. Though the lymphocytes in the radiated groups gradually commenced to return to normal, they had not done so at the end of two weeks after the tumor had been inoculated, a time period sufficient for this tumor to have become established if it were going to grow.

The blood from the normal untreated controls for the whole experiment (group E) showed a slight initial fall in the lymphocytes, though these animals had received no *x*-ray or other treatment, followed by a marked rise in the number of lymphocytes. This lymphocytosis according to some of the current theories should have caused the tumors, if not to regress, at least not to continue to grow so well. Actually, however, they behaved as the Flexner-Jobling rat carcinoma does under normal conditions in any susceptible strain; and the tumors grew rapidly and well.

In this series, groups C and D, in which the percentage of takes was 10.5 and 4.8 respectively, might at first glance seem to indicate a marked increase in the number of takes after x-ray treatment. If the figures are studied, however, it will be seen

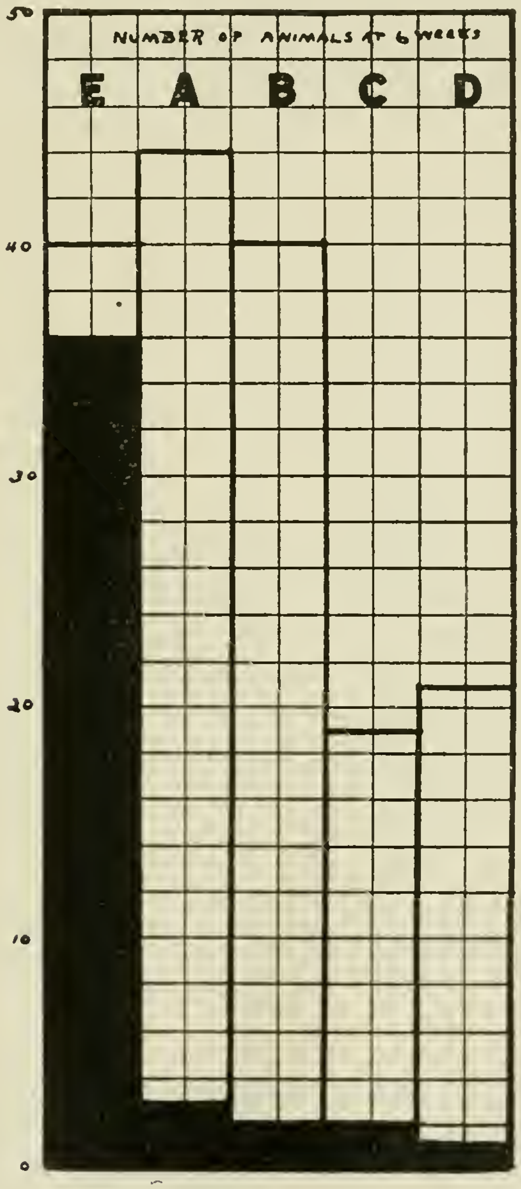

FIG. 2

that two takes to seventeen negatives in group C and one take to twenty negatives in group D do not offer a difference large enough to permit of the drawing of any deductions, such fluctuations being frequent in different series of inoculations of this tumor.

In another series the same experiment was duplicated, and as further controls, a few animals from the resistant strain, which had been previously immunized with rat embryo skin emulsion and then proved to be immune to Flexner-Jobling rat carcinoma by previous inoculations, were used. With this group it was thought advisable not to allow the lymphocytes in the radiated animals to return to normal; therefore, whenever they showed a tendency to do so, the reducing exposures to x-ray were repeated, so that in six weeks, they had received three separate series of reducing exposures to x-ray. The results obtained, however, were in accord with those previously described, for at the end of six weeks, in the animals which had previously been proved immune (groups C, D, F, and G) there were no takes in either the radiated or the non-radiated series (Table 2).

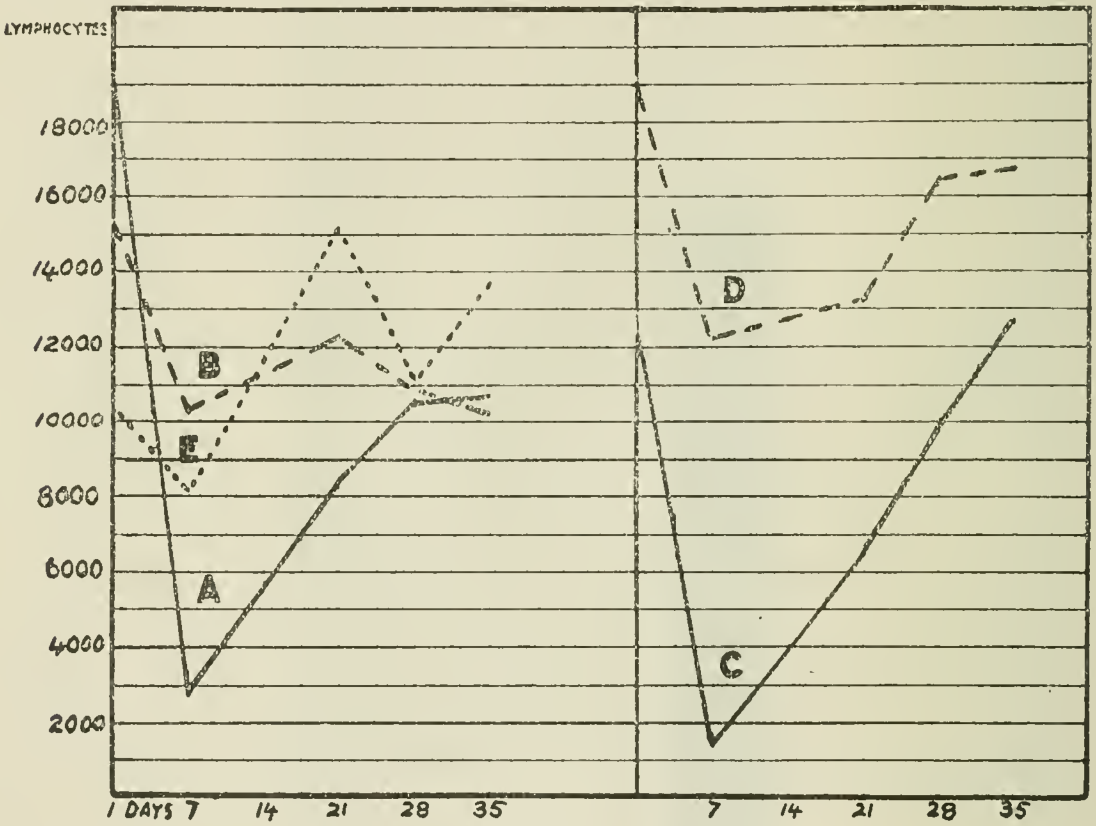

FIG. 3

In groups A and B, in which A had been given x-ray for three series of irradiations and B had had no treatment, the difference is so small as to be negligible. The lymphocyte counts of this series were taken at weekly intervals (text-figure 4) and show that by repeated radiations it is possible to keep the lymphocytes in the circulating blood very much below the normal figure.

TABLE 2

F R C / 67 G	NUMBER OF RATS	POSITIVE	NEGATIVE	PERCENTAGE OF TAKES	PERCENTAGE NEGATIVE	EXPLANATION
Group A	15	1	14	6.7	93.3	Resistant stock; no previous inoculation; given reducing exposures of *x*-ray
Group B	17	1	16	5.8	94.2	Resistant stock; no previous inoculation; no *x*-ray
Group C	4	0	4	0	100.0	Resistant stock; previously inoculated unsuccessfully with F. J. R. C.; given reducing exposures of *x*-ray
Group D	10	0	10	0	100.0	Resistant stock; previously inoculated unsuccessfully with F. J. R. C.; no *x*-ray
Group E	13	9	3	69.3	30.7	Ordinary stock. Control for experiments
Group F	19	0	19	0	100.0	Resistant stock; previously treated with embryo skin; inoculated unsuccessfully with F. J. R. C.; given reducing exposures of *x*-ray
Group G	22	0	22	0	100.0	Resistant stock; previously treated with embryo skin; inoculated unsuccessfully with F. J. R. C.; no *x*-ray

In order to see whether by any chance a sarcoma would be influenced in any different way by the reduction of the lymphocytes in the circulating blood, the same experiment was repeated, using a rat sarcoma (R 10 of the Crocker Fund series). The animals were divided into groups as in the previous experiments; and the blood was counted before and after *x*-ray treatment and at frequent intervals thereafter.

The number of takes at the end of six weeks is shown in table 3. In this series as in the previous ones the composite blood curve (text-figure 5), showed a marked reduction in the number of circulating lymphocytes, but with slightly more rapid return towards the normal. In groups C and D unfortunately a majority of the rats died from some unknown cause. On that account, therefore, the experiment was repeated on a somewhat larger scale, with the results shown in table 4. Here, however, although several months and a number of generations of the tumor had elapsed between the two series of experiments, the results were the same. When the resistant animals were rendered immune by previous doses of tumor and were then given reducing doses of x-ray, there was no difference between the treated animals and the controls as regards the number of growths at the end of five weeks. In both groups the results were 100 per cent negative. In all the series, however, except in the case of the Jensen rat sarcoma, when the resistant stock had not been rendered immune by previous inoculations the animals treated with x-ray showed a slightly higher percentage of takes than did the animals which had not been given x-ray. It would seem, however, that the importance of this experiment lies in the results obtained from the *immune* animals, for it is only from these that we can draw accurate conclusions as to the rôle played by the lymphocyte in tumor production. In going over the chart

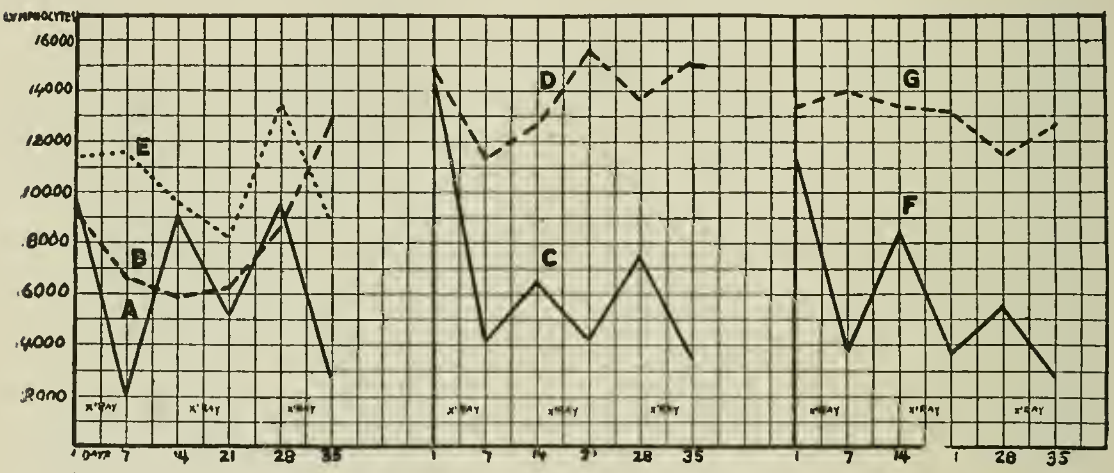

Fig. 4

TABLE 3

$\frac{\text{R 10}}{\text{23 E}}$	NUMBER OF RATS	POSITIVE	NEGATIVE	PERCENTAGE OF TAKES	PERCENTAGE NEGATIVE	EXPLANATION
Group A	21	3	18	14.2	85.7	Resistant stock; no previous inoculation; given reducing exposures of *x*-ray
Group B	24	3	21	12.5	87.5	Resistant stock; no previous inoculation; no *x*-ray
Group C	8	0	8	0	100.0	Resistant stock; previously inoculated unsuccessfully with R 10; given reducing exposures of *x*-ray
Group D	3	0	3	0	100.0	Resistant stock; previously inoculated unsuccessfully with R 10; no *x*-ray
Group E	22	21	1	95.4	4.5	Ordinary stock. Control for experiment

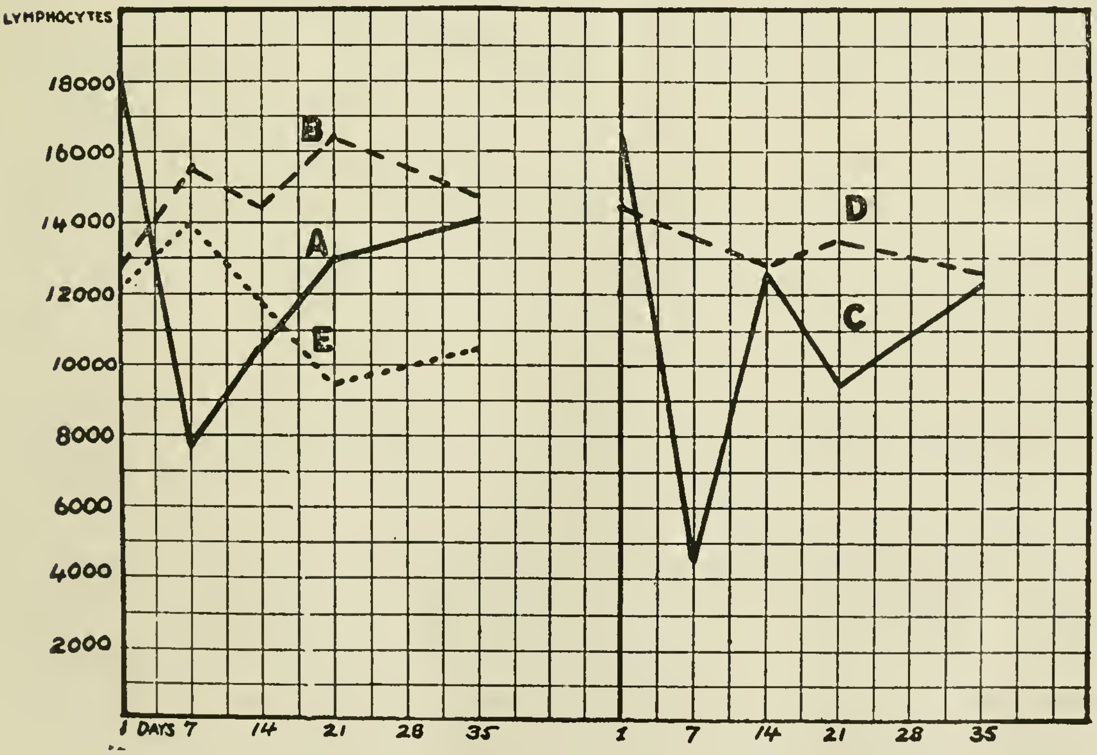

FIG. 5

books in the Crocker Laboratory for the last six years, there was found in all our series of the Jensen rat sarcoma a variation in the number of takes. The difference in the takes in groups A and B, therefore, though it may look suggestive, certainly does not warrant the drawing of any deductions, for greater fluctuations occurred in routine transplantations.

In this sarcoma series, as in the carcinoma, the results do not show the effects of *x*-radiation reported by Murphy and Morton or by Mottram and Russ (4). The latter investigators found, during a series of experiments with the Jensen rat sarcoma, that animals which had been previously proved immune to inoculations of that tumor would, when given sufficiently large doses of *x*-ray to reduce the lymphocyte count markedly, permit the growth of the Jensen rat sarcoma. They, however, gave their *x*-ray doses in one prolonged treatment rather than repeated over a protracted period. They deduce from their experiments that some of the original grafts persisted for a longer time in the irradiated animals than in the controls, and that when the tumor did grow it

TABLE 4

$\frac{\text{R 10}}{\text{30 B}}$	NUMBER OF RATS	POSITIVE	NEGATIVE	PERCENTAGE OF TAKES	PERCENTAGE NEGATIVE	EXPLANATION
Group A	66	42	24	63.6	36.4	Resistant stock; no previous inoculation; given reducing exposures of *x*-ray
Group B	68	37	31	54.5	45.5	Resistant stock; no previous inoculation; no *x*-ray
Group C	53	0	51	0	100.0	Resistant stock; previously inoculated twice unsuccessfully with R 10; given reducing exposures of *x*-ray
Group D	65	0	65	0	100.0	Resistant stock; previously inoculated twice unsuccessfully with R 10; no *x*-ray
Group E	60	59	1	98.3	1.7	Ordinary stock. Control for experiment

was delayed in making its appearance and almost invariably receded; but in our series this also occurred when *x*-ray had not been given, and the difference in the tumors which survived and grew in groups A and B show nothing to indicate any marked variation in favor of those whose lymphocytes had been reduced by *x*-ray radiation. The number of animals used by them is too small to permit the drawing of general conclusions as to the relationship between the *x*-rays and the presence or absence of immunity, except with the Jensen rat sarcoma and in the particular growth phase at the time the work was done. For example, the record charts of the Crocker Fund show that an occasional rat immune to inoculation with this tumor may give a good growth on reinoculation. In this laboratory, also, we do not find that the Jensen rat sarcoma when inoculated into rats grows almost invariably, as it does in England; and a careful analysis of 5543 animals extending over a period of six years shows the average number of takes to have been only 71 per cent. The number of takes varied greatly according to the months, from 45 per cent in July to 77 per cent in August.

In order, therefore, to test the results of Mottram and Russ, and to add another type of sarcoma to our experimental series, a group of rats was given *x*-ray treatment under conditions similar to those of the foregoing experiments, and then inoculated with the Jensen rat sarcoma. The results are shown in table 5. In group A, comprising animals of resistant stock whose lymphocytes had been reduced by exposures to *x*-ray, there were fewer takes than in group B, made up of animals of resistant stock which had not been exposed to *x*-ray; whereas in the immune animals the results were 100 per cent negative, whether they had been exposed to reducing doses of *x*-ray or not. The blood pictures were the same as those shown in the above charts, and have, therefore, been omitted. In all these experiments, it was found, just as was reported by Mottram and Russ, that occasionally a small tumor in the radiated animals would persist longer than one in the controls; but even when the lymphocytes were kept low and not allowed to return to normal, the tumor ultimately disappeared. It may be that the introduction of a graft of tumor into an animal acts as an antigen, producing a small amount of a cytotoxic antibody, and thus causes regression of small, poorly established tumors; and it is also possible that the action of the x-ray on such animals is to hold the production of the antibody in check, as has been shown by Hektoen. This inhibition of antibody formation may permit a pin-point or small growth to remain for a time; but from our results and those of others it does not seem that this persistence is due to the lowering of the lymphocytes in the blood, as ultimately these small growth disappear despite the lowering of the cell count.

TABLE 5

JRS / 95 A	NUMBER OF RATS	POSITIVE	NEGATIVE	PERCENTAGE OF TAKES	PERCENTAGE NEGATIVE	EXPLANATION
Group A	36	15	21	41.7	58.3	Resistant stock; no previous inoculation; given reducing exposures of x-ray
Group B	61	30	31	49.1	50.9	Resistant stock; no previous inoculation; no x-ray
Group C	19	0	19	0	100.0	Resistant stock; previously inoculated twice unsuccessfully with J. R. S.; given reducing exposures of x-ray
Group D	43	0	43	0	100.0	Resistant stock; previously inoculated twice unsuccessfully with J. R. S.; no x-ray
Group E	59	41	18	69.8	30.2	Ordinary stock. Control for experiment

SUMMARY

In animals which are naturally resistant or artificially immune to certain strains of rat carcinoma and sarcoma, a marked reduction in the circulating lymphocytes produced by x-ray treatment is not followed by an appreciable decrease in their immmnity to tumor inoculation.

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FLUCTUATIONS IN INDUCED IMMUNITY TO TRANSPLANTED TUMORS

F. D. BULLOCK AND G. L. ROHDENBURG

From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director

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Investigators in cancer research who have performed any large number of experiments in which an attempt has been made to induce immunity against transplantable animal tumors have probably been impressed by the variations in the percentages of actual immunity produced by the methods commonly employed. Perhaps it has been their experience to obtain wide differences in their results at different periods in the life of a particular tumor even after using the same technic and immunizing material. If they have had occasion to review the published experiments in the same field they will have observed that these variations have also occurred in the work of other men.

As illustrative of these variations, we present Chart 1, which depicts graphically the results obtained in rats immunized with 0.05 gram of rat fetal skin, 0.05 gram of rat spleen emulsion and 1 cc. of rat blood cells respectively, and inoculated ten days later with the Flexner-Jobling rat carcinoma in dosage of 0.003 gram.[1] The relatively small dose of immunizing material was intentionally used to secure, if possible, a more delicate reaction. It will be noted that an immunity varying from 3 to 70 per cent was obtained, and that these variations occurred irrespective of the kind of immunizing material used. In these groups there was no relationship between the percentage of natural immunity as present in the control groups and that of induced immunity in the immunized groups.

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[1] In previous publications from the Imperial Cancer Research Fund and from this laboratory, the inoculation dose, when the needle method is used, has been estimated as 0.01 or 0.02 gram; but such grafts have recently been found, as a matter of fact, to weigh about 0.002 and 0.003 gram respectively.

It is, of course, obvious that if variations in induced immunity are at all common their occurrence introduces a grave factor of error in many of the experiments in which immunity plays a part. It is possible, for example, for one investigator by using one kind of immunizing material to obtain no immunity against a particular tumor, while another by employing the same kind of immunizing material and tumor strain may obtain a high percentage of immunity. The publication of the two experiments with directly opposite results would tend to create confusion unless the occurrence of such extraordinary fluctuations were fully appreciated.

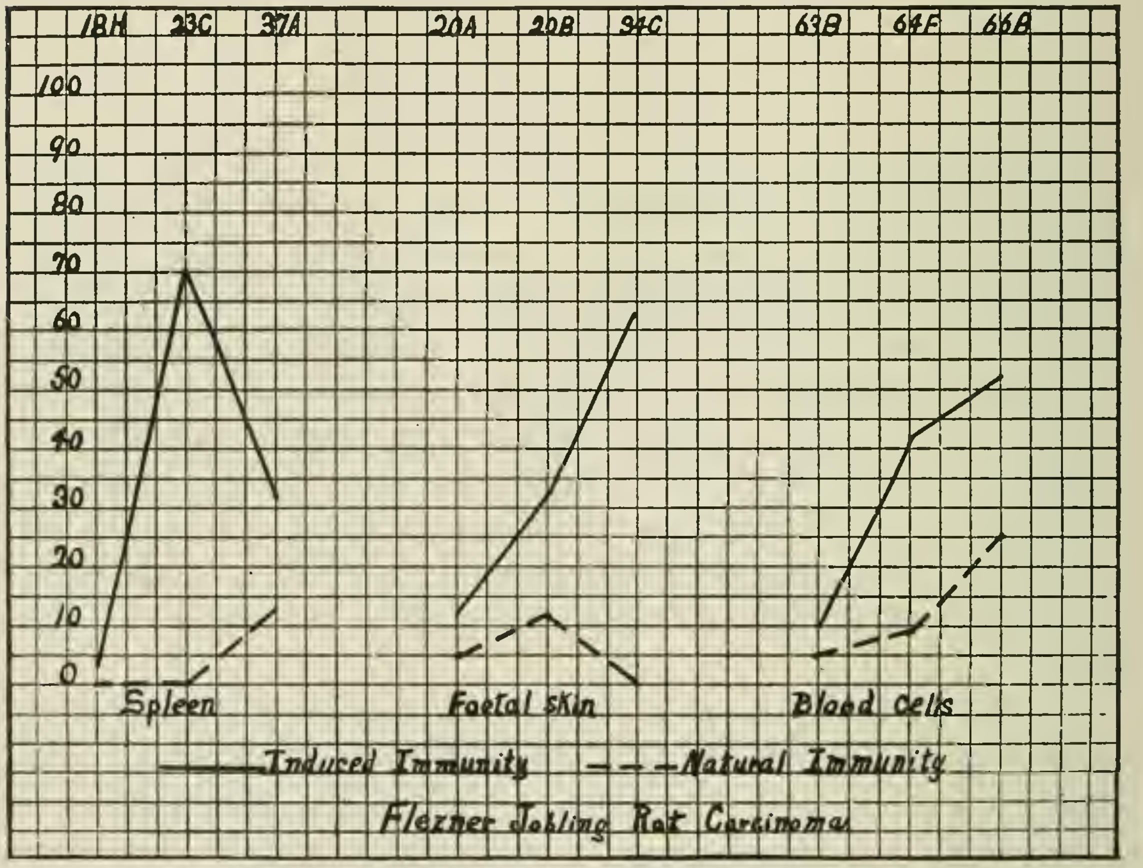

Chart 1. Fluctuations in Percentages of Induced and Natural Immunity with Flexner Jobling Rat Carcinoma

The object of the experiments recorded in this paper was to establish the fact that fluctuations in induced immunity are a frequent occurrence, and to determine, if possible, the factors responsible for them so that in the future they might be eliminated. In our investigations, three tumor strains were used; the Flexner-Jobling rat carcinoma, the Buffalo rat sarcoma, and the English mouse carcinoma 63.[2]

In order to exclude as far as possible the factor of variation in technic, a standard was decided upon and rigidly followed. Immunity was induced by subcutaneous injection of homologous splenic pulp emulsion in dosage of 0.07 gram. Ten days after immunization, the animals were inoculated subcutaneously with 0.003 gram of the tumor strain. Immunization and inoculation were performed by the same person in all experiments in order to eliminate the factor of individual variation in technic. The tumors used for inoculation were derived whenever possible from the most recent generation and were eighteen days old. The growth of the tumor was charted for the first time ten days after inoculation and weekly thereafter. The percentages are based on conditions as present on the twenty-fourth day of tumor growth.

Twenty-four animals were used in each series, the controls being a similar number of normal non-immunized animals derived from the same stock. In the entire experiment, animals of about the same age, development, and state of health were chosen.

Chart 2 presents in graphic form the percentages of immunity obtained with the Buffalo rat sarcoma by following the outlined technic. The percentages varied in different generations from 18 as a low, to 95 as a high point. Although the percentages of natural immunity also varied the variations bore no relation to those of induced immunity.

While sarcoma is notoriously difficult to immunize against, the criticism may perhaps be advanced that the fluctuations noted are dependent upon the health of the animals, it being well known that animals in poor health are less susceptible to tumor inoculation than are healthy animals. One index of the health of any given group of organisms is the mortality rate. This rate has been plotted as a curve in this and in the succeeding series to be later described. It is true that with the Buffalo tumor, with a mortality of 10 per cent, the immunity induced was 18 per cent and that when the mortality was 42 per cent the immunity reached 95 per cent. For this series, and for this series only, it is apparently true that when the animals were healthy, as evidenced by a low mortality, the percentage of

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[2] This laboratory is indebted to Dr. Murray, Director of the Imperial Cancer Research Fund of London, England, for the last named tumor strain.

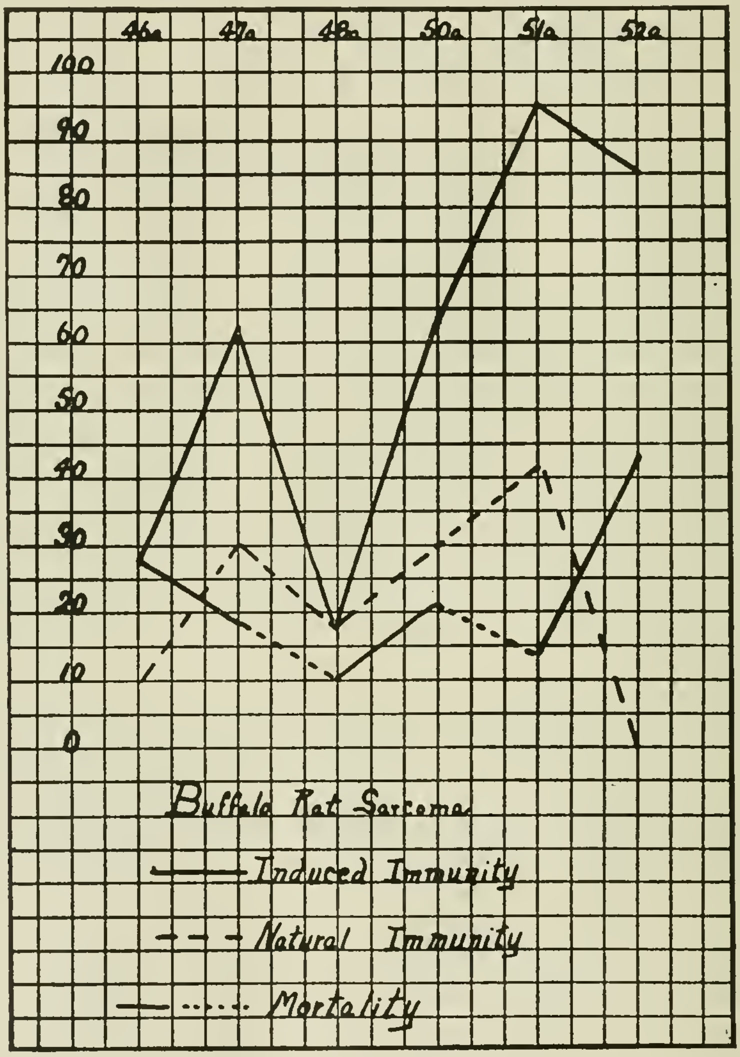

Chart 2. Fluctuations in the Percentages of Induced and Natural Immunity of the Buffalo Rat Sarcoma

induced immunity was low, while at another time when the health of the animals was poor, with a high mortality, the immunity was also high.

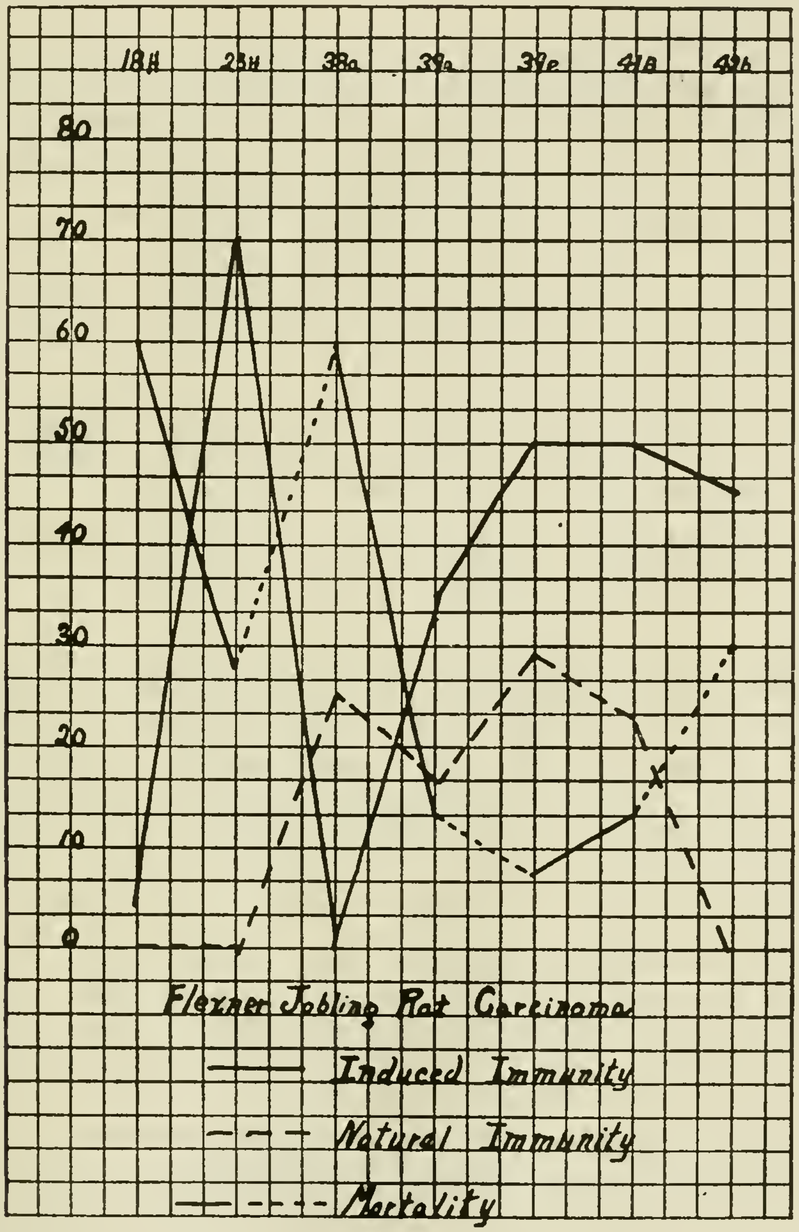

CHART 3. FLUCTUATIONS IN THE PERCENTAGES OF INDUCED AND NATURAL IMMUNITY OF THE FLEXNER JOBLING RAT CARCINOMA

When the Flexner-Jobling tumor was used, variations again occurred as is shown in chart 3. In this series the low point was zero while the high point was 70 per cent. The mortality percentages in this series, however, present the directly opposite relationship apparent with the Buffalo tumor, for on two occasions when the death rate was 60 per cent, in other words, when the health of the animals was poor, there was practically no immunity obtained.

Pitzman (1), in explanation of these fluctuations, has advanced the hypothesis that immunity is due to bacterial contamination of either the tumor or the immunizing material, or possibly of both. This suggestion has been, however, shown to be without foundation by Tyzzer (2) and Woglom (3). But to rule out any possible source of error two generations in which the mouse carcinoma 63 was employed were immunized with mouse fetal skin emulsion instead of splenic tissue, and cultures were made of the immunizing material and of the tumor tissue. All the cultures proved sterile. Bacterial contamination can, therefore, be eliminated. Nevertheless, as is shown in chart 4, there was a marked variation in the percentage of immunity obtained.

One other factor that remains to be considered is the purity of breed of the animals used. The influence of this factor upon the growth of first and even subsequent transplants of tumor is well known; for example, tumors from Danish mice may not grow in mice bred in Germany. It was thought possible that a similar circumstance might explain the variations encountered in inducing immunity. To control this element the mouse tumor 63, which, as we have stated before, is of English derivation, was inoculated into pure bred English mice, immunity being induced in the usual manner by means of mouse spleen emulsion. As will be noted in chart 5, the variations encountered were slight, the high point being 100, the low point being 88 per cent. Variations of but twelve points in a biological experiment of this kind can be disregarded, as they fall within the limits of error.

It is evident from the data presented that variations of marked degree do occur in the percentages of induced immunity obtained after the commonly accepted methods of procedure. The importance of the recognition of these fluctuations is self-evident, since their occurrence markedly impairs the value of any deductions from experiments in which they have not been considered and eliminated.

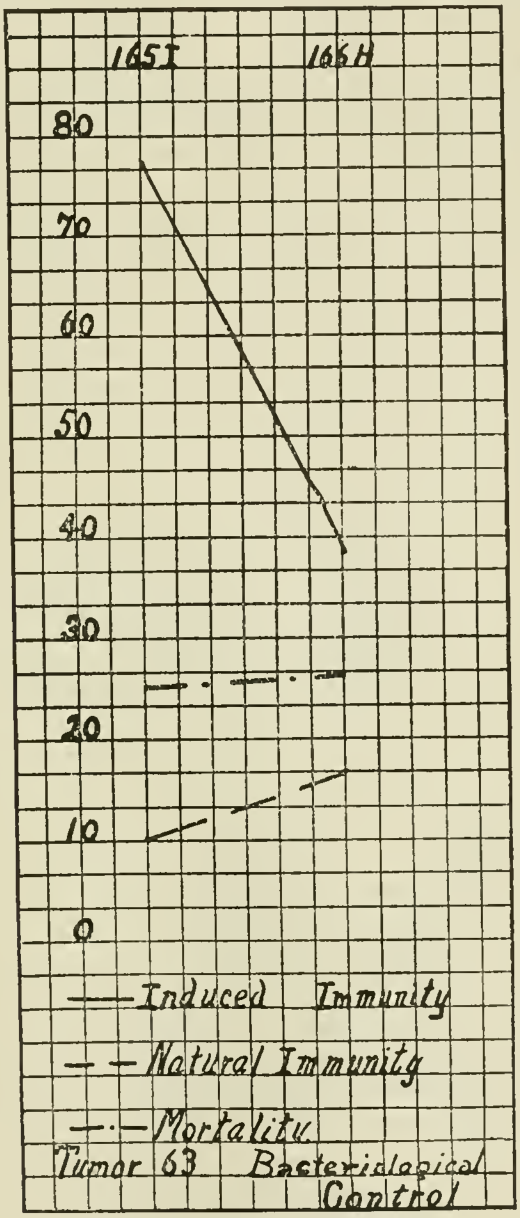

Chart 4. Variations in Induced Immunity with Tumor 63, When Both Tumor and Immunizing Material Are Shown to Have Been Sterile

The factor responsible for these variations is at first glance not quite evident. Variations in technic, particularly in minor alterations in the dosage of the immunizing material, have been eliminated. Bacterial contamination, with a possible destruction of the inoculated fragments of tumor, is also not sufficient to explain the variations noted, since fluctuations occurred when both immunizing tissue and tumor were sterile. The type of tissue used for immunization cannot be held responsible, for the variations were noted with blood cells, spleen emulsion, and fetal skin emulsion. Epithelial and connective tissue tumors of both the rat and the mouse showed the variations. However, when the purity of breed of the experimental animals was assured then the variations were of such minor type that they

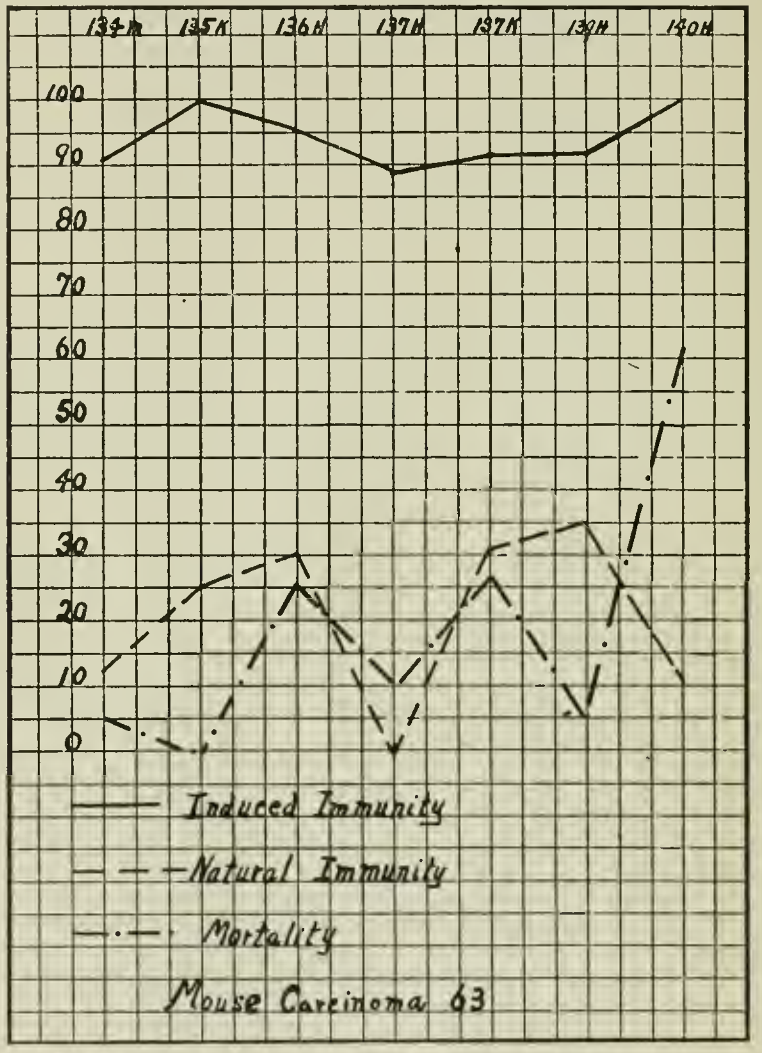

CHART 5. FLUCTUATIONS IN THE PERCENTAGES OF INDUCED AND NATURAL IMMUNITY OF TUMOR 63

could be disregarded. Russell (4) obtained fairly constant results in his published experiment in induced immunity by using a pure strain of mice. The most plausible explanation, therefore, is that the biological relationship between tumor, immunizing material, and host is a very delicate one and that unrecognizable differences between different strains of animals are sufficient to create the wide variations noted.

In any experiment in which immunity plays a part these variations should be eliminated or rendered as small as possible by first transplanting the tumor for a number of successive generations into a pure strain of animals and then using animals of the same strain for the immunity experiments. It is only by doing this that accurate deductions from experimental results will become possible.

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FLUCTUATIONS IN CONCOMITANT IMMUNITY

F. D. BULLOCK AND G. L. ROHDENBURG

From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director

Received for publication, July 2, 1919

The variations in the percentage of induced immunity which we obtained by the methods commonly used in immunizing animals against transplanted tumors have been discussed in another publication (1). In the present paper it will be shown that fluctuations of a similar character occur in concomitant immunity, following a previous inoculation with either a receding or a progressively growing tumor.

In investigations which have been recorded elsewhere (2) the action of degenerative tumor products upon immunity was studied, it being our original intention in planning these experiments to see if tumors which do not produce concomitant immunity could be made to do so by an increase in the products of tumor degeneration. In this connection it will be remembered that Russell (3) has divided tumors into two classes, those which immunize against subsequent grafts of a tumor of the same strain (concomitant immunity) and those which do not. He presented the mouse carcinomata Twort and 63[1] as typical of those which do not produce concomitant immunity. The results of our experiments referred to above are of interest in this connection because of the fact that in our hands these two tumor strains did produce concomitant immunity. Chart 1 copied from Russell's article shows his results with the Twort tumor, while Chart 2 depicts our results with the same strain. Chart 3 is a reproduction of Russell's results with tumor 63, while charts 4 *a*, 4 *b* and 4 *c* represent our results with the same strain. It will

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[1] This laboratory is indebted to Dr. Murray, Director of the Imperial Cancer Research Fund of London, England, for the tumor strains Twort, 63, and 206.

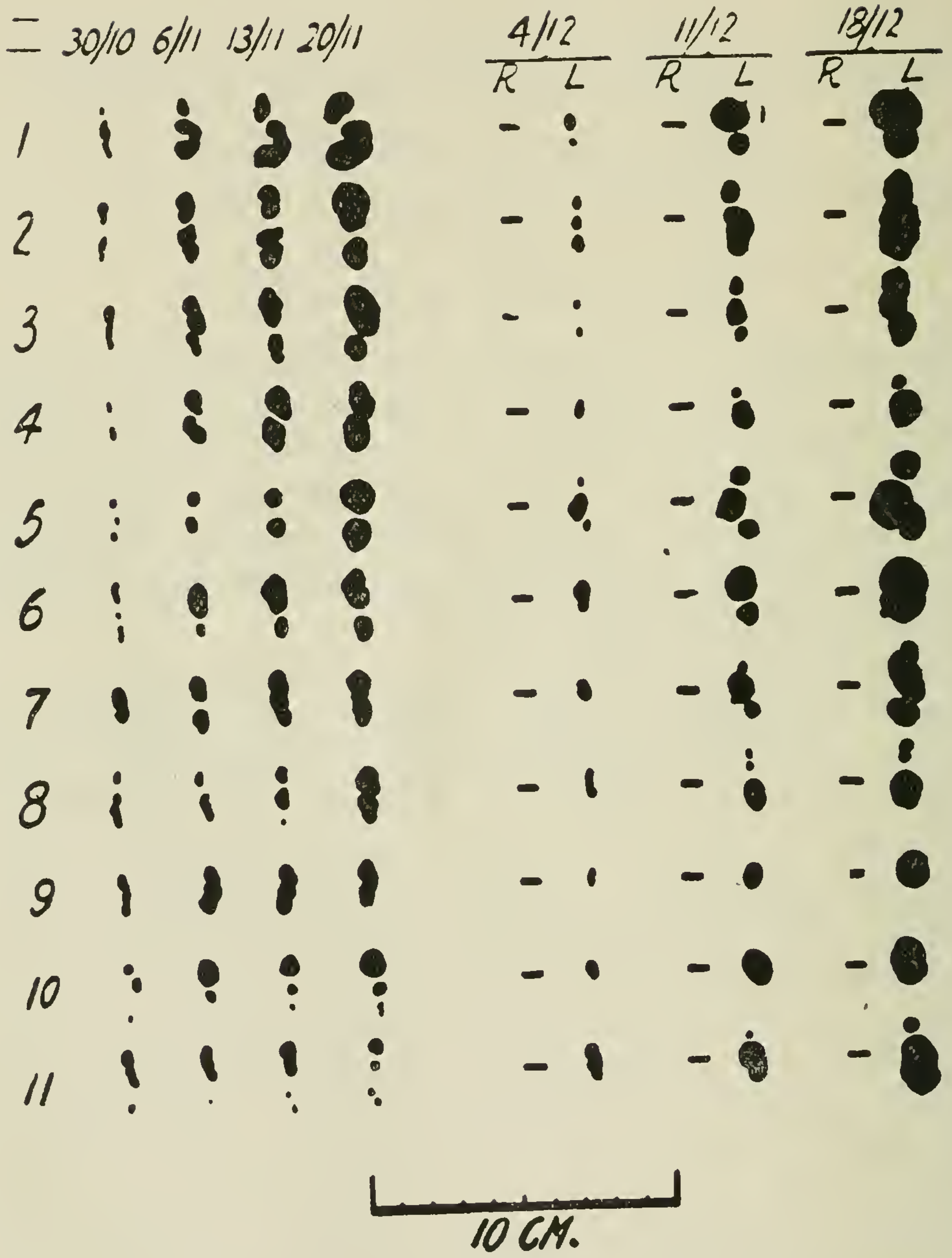

Chart 1. Twort Tumor in Non-Immunizing Phase (After Russell)

Mice inoculated in right axilla 20/10. Tumors excised 22/11 and the animal reinoculated in left axilla 24/11.

be noted that our results do not agree with those obtained by Russell.

Prime, of this laboratory, unaware of our experiments and while working on another problem, also obtained results at variance with those of Russell while using the British mouse carcinoma 206 as an immunizing agent. According to Russell (4) this tumor strain immunizes against a subsequent inoculation of the British mouse carcinoma 63 and the records of his experiment show an immunity of 100 per cent. Prime, on the contrary, in an experiment heretofore unrecorded (chart 5), obtained but 23 per cent immunity in one generation and 30 per cent in the next, a variation of approximately 70 from Russell's figures.

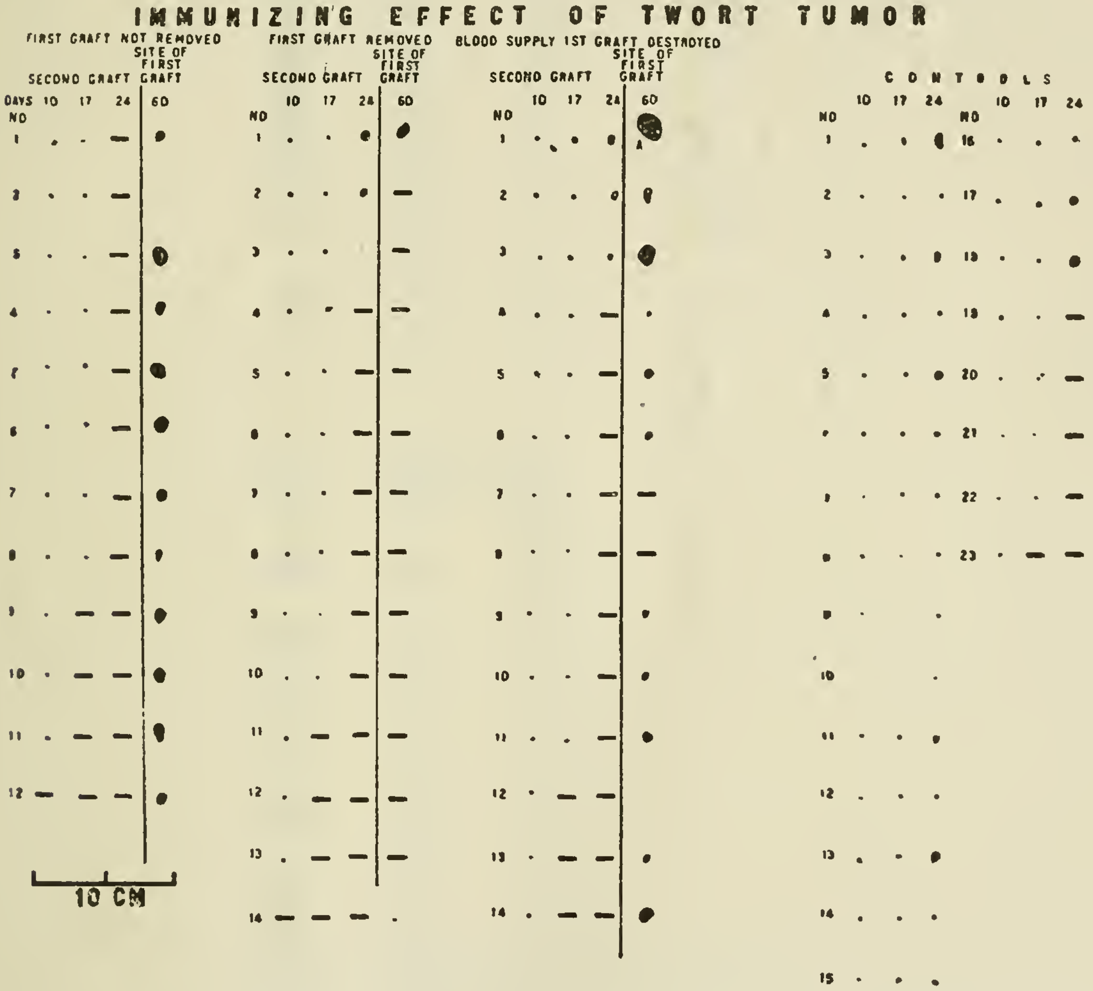

Chart 2. An Experiment with Twort Tumor Showing a High Percentage of Concomitant Immunity

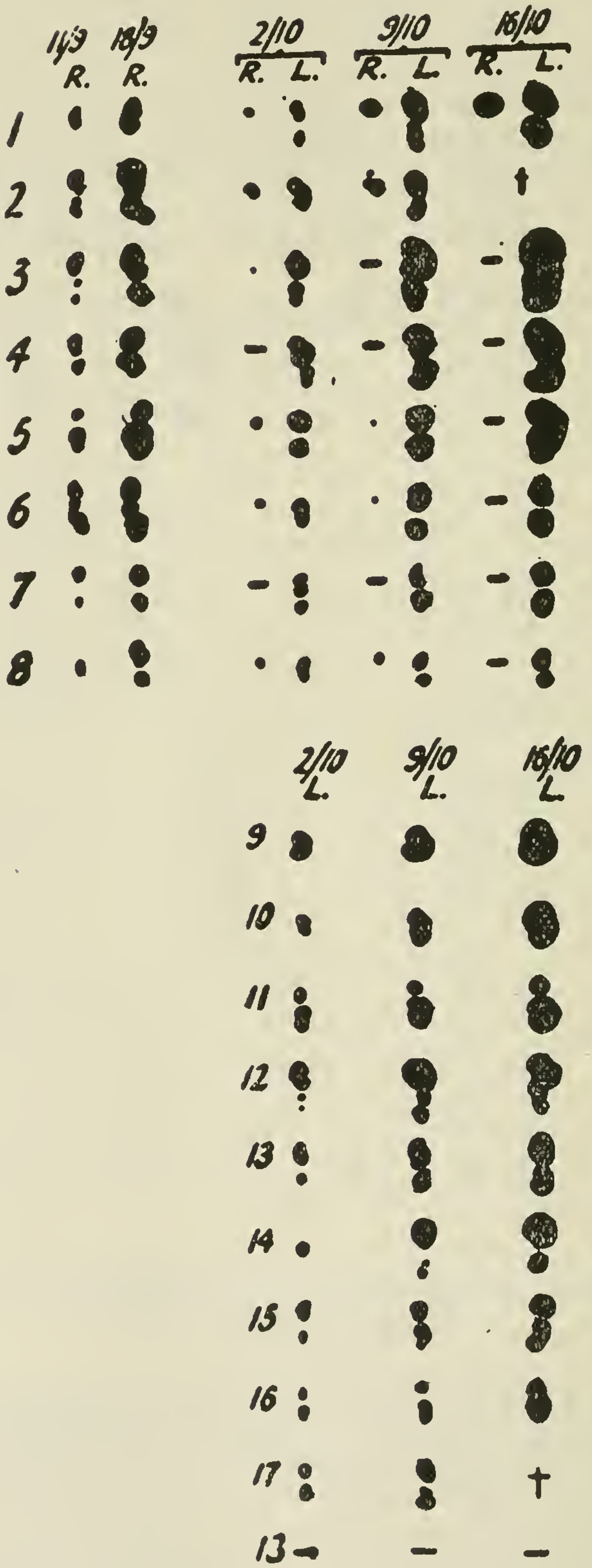

Chart 3. Tumor 63 in Non-Immunizing Phase (After Russell)

Mice 1 to 8 inoculated in right axilla 31/8. Tumors excised 19/9 and the mice reinoculated in left axilla 21/9. Mice 9 to 18 controls for second inoculation.

The objection may be raised that these are isolated occurrences readily explained by the fact that English tumors have been transplanted into animals of American stock. In answer to this objection, we present similar results which were obtained with the Buffalo rat sarcoma. Chart 6 shows this tumor in a phase when it did induce concomitant immunity, while chart 7 shows it at a time when that power was lost.

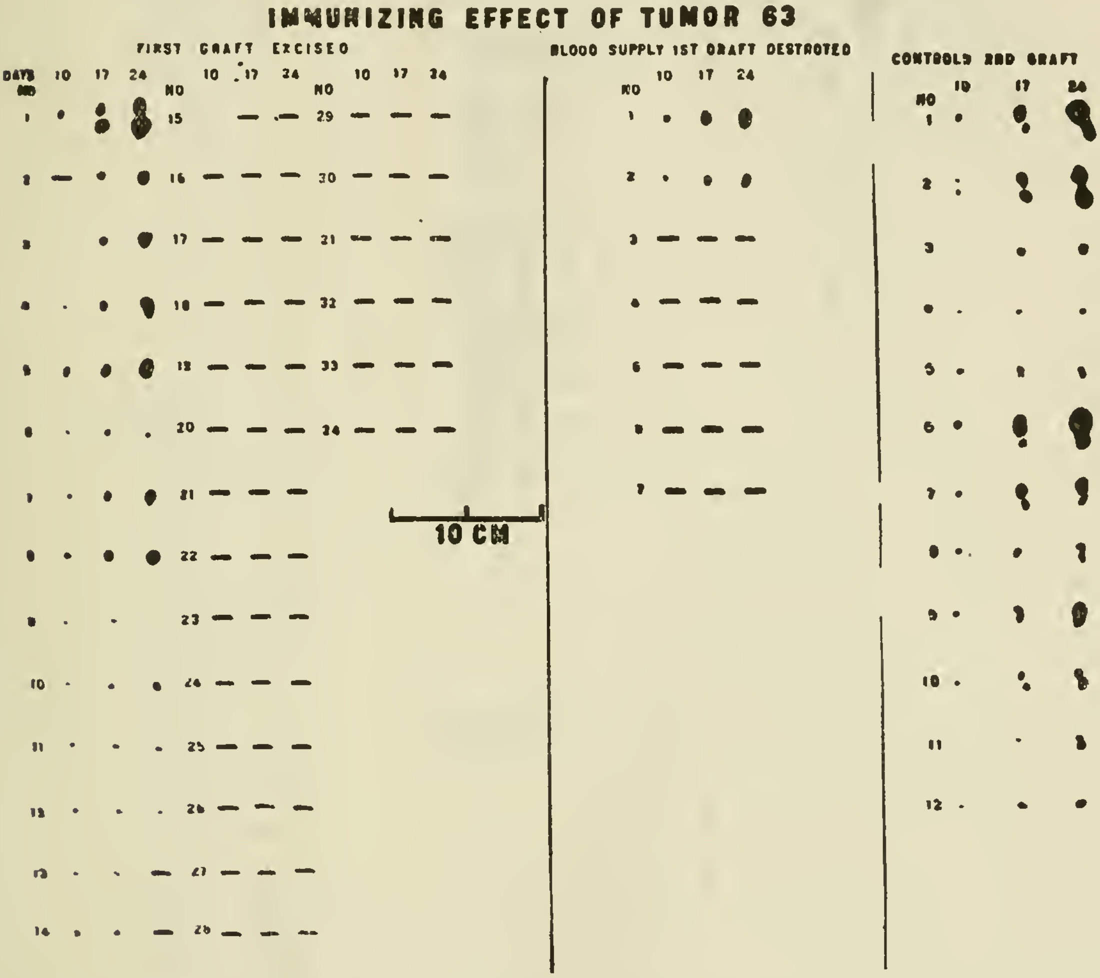

Chart 4 a. An Experiment in Which Tumor 63 Gave Rise to a High Degree of Concomitant Immunity

The above examples have been cited to show that fluctuations in concomitant immunity do occur. The experiments recorded in this paper were planned to determine the frequency of this occurrence and to discover, if possible, the factor or factors which are responsible for these variations. As in our experiments in induced immunity, three tumor strains were used, the Buffalo rat sarcoma, the Flexner Jobling rat carcinoma, and the English mouse carcinoma 63.

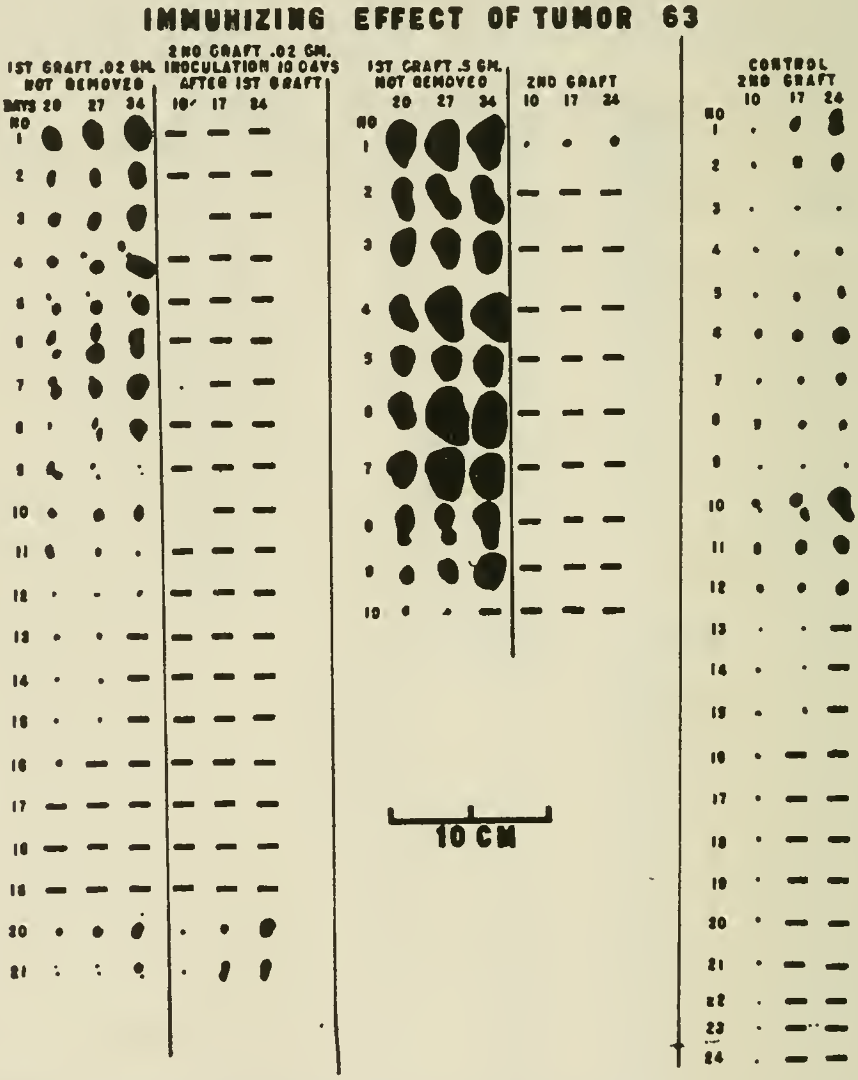

CHART 4 B. ANOTHER EXPERIMENT IN WHICH TUMOR 63 GAVE RISE TO A HIGH DEGREE OF CONCOMITANT IMMUNITY

To eliminate variations due to differences in technic, a standard was decided upon and all inoculations were performed by the same operator. Animals were inoculated with 0.003 gram[2] of

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[2] In previous publications from the Imperial Cancer Research Fund and from this laboratory, the inoculation dose, when the needle method is used, has been estimated as 0.01 or 0.02 gram; but such grafts have recently been found, as a matter of fact, to weigh about 0.002 and 0.003 gram, respectively.

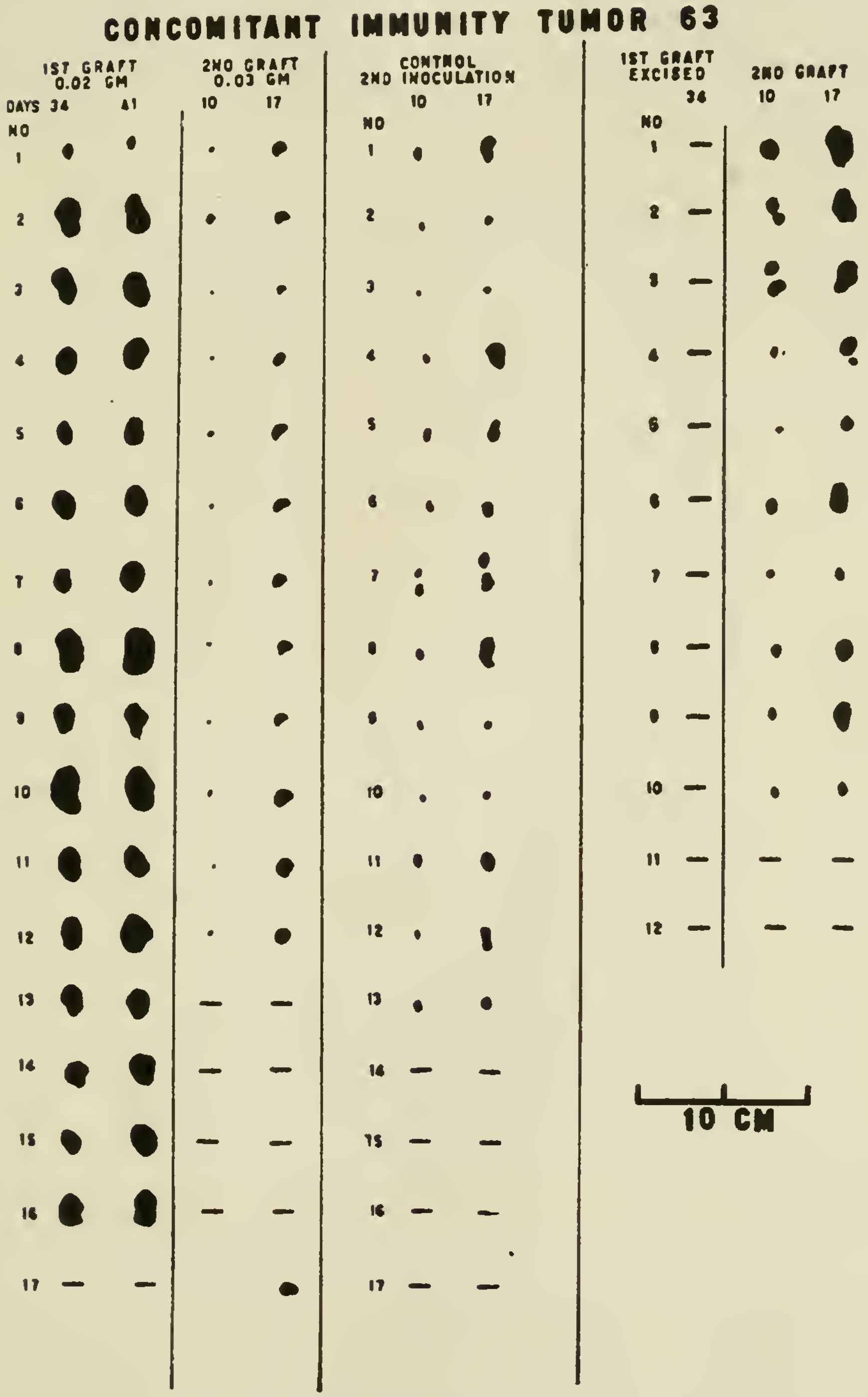

Chart 4 c. An Experiment in Which Tumor 63 Closely Followed Russell's Experience the given tumor strain, the tumors for inoculation being derived, whenever possible, from the preceding generation, and being 18 days old. Eighteen days after inoculation, the animals were reinoculated in the tissues of the opposite side of the body with a similar dose of tumor of the same strain. The tumor used for the second inoculation was selected from the tumors resulting from the first inoculation. Twenty-four animals were used for each generation, and each set of inoculations was controlled by a normal group of 24 animals. In the entire experiment animals of about the same age, development, and state of health were chosen. The tumors were charted for the first time ten

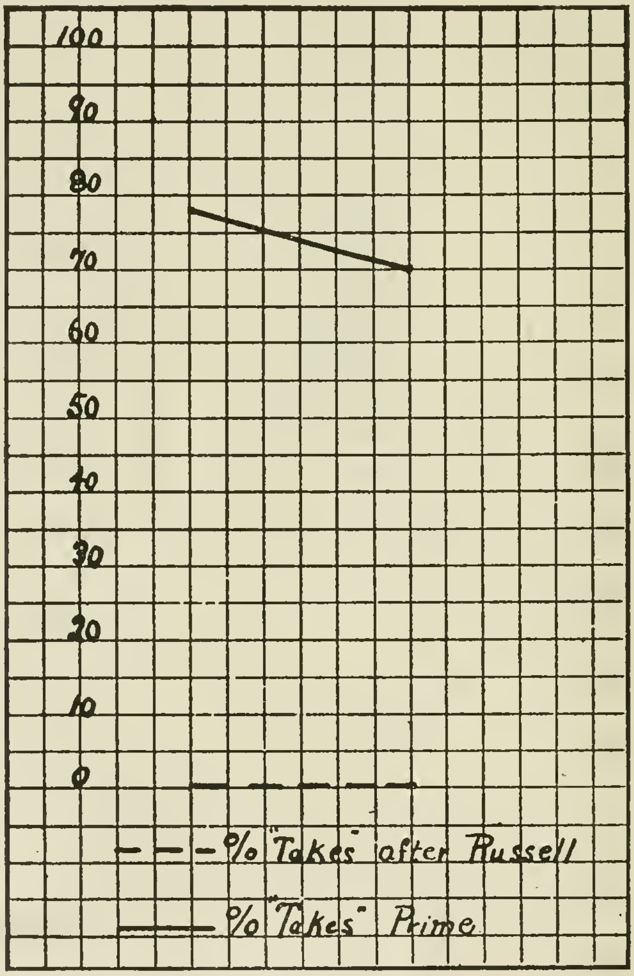

Chart 5. A Comparison of the Percentages of Takes Obtained by Russell and by Prime Using the Tumor 206 and 63

days after inoculation and weekly thereafter, and our figures are based on the condition present on the twenty-fourth day of growth of the second graft.

Concomitant immunity, as observed through six generations of the Buffalo rat sarcoma (chart 8), ranges between 100 per cent and 65 per cent, a variation of 35 per cent. No relationship is apparent between the curves of concomitant and those of induced or natural immunity in the same generations (chart 9).

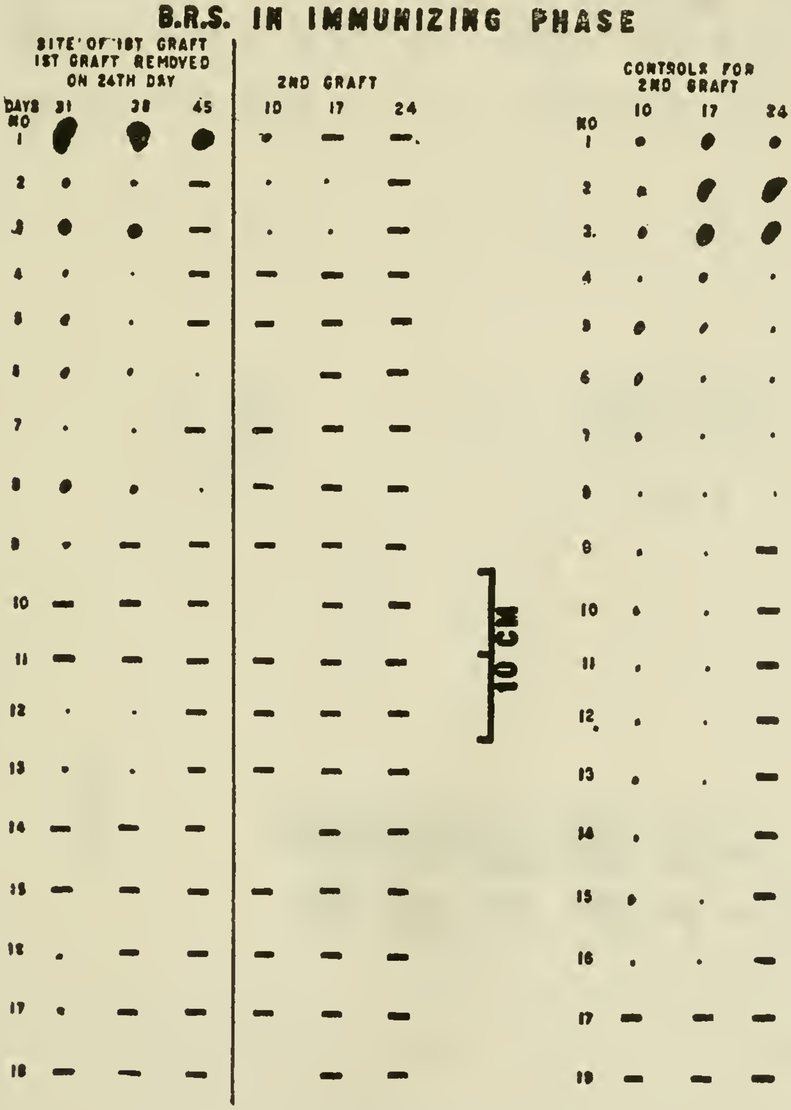

CHART 6. BUFFALO RAT SARCOMA IN A PHASE WHERE IT INDUCED CONCOMITANT IMMUNITY

Although the power to produce concomitant immunity is not

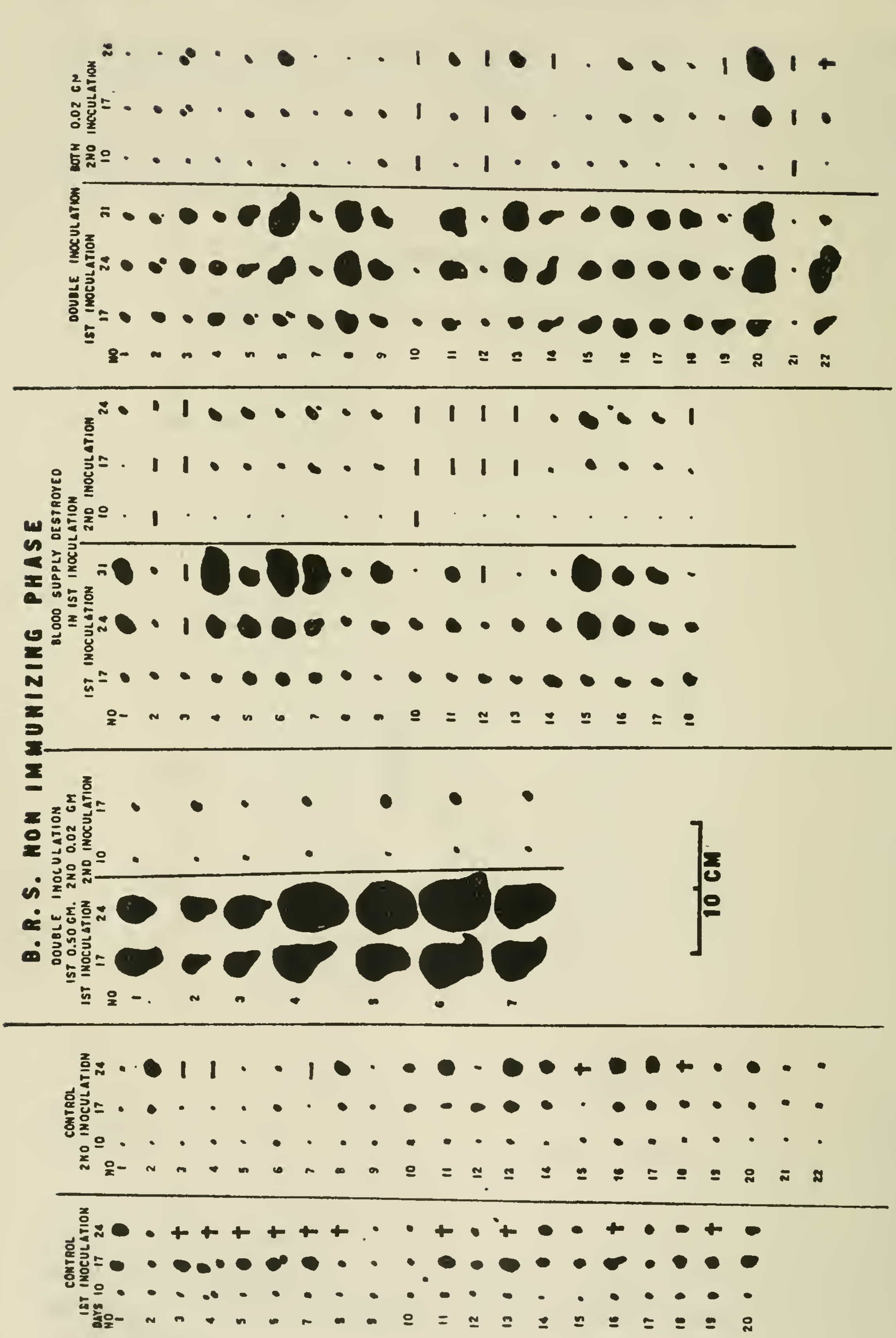

Chart 7. Buffalo Rat Sarcoma in a Phase Where it Did Not Induce Concomitant Immunity

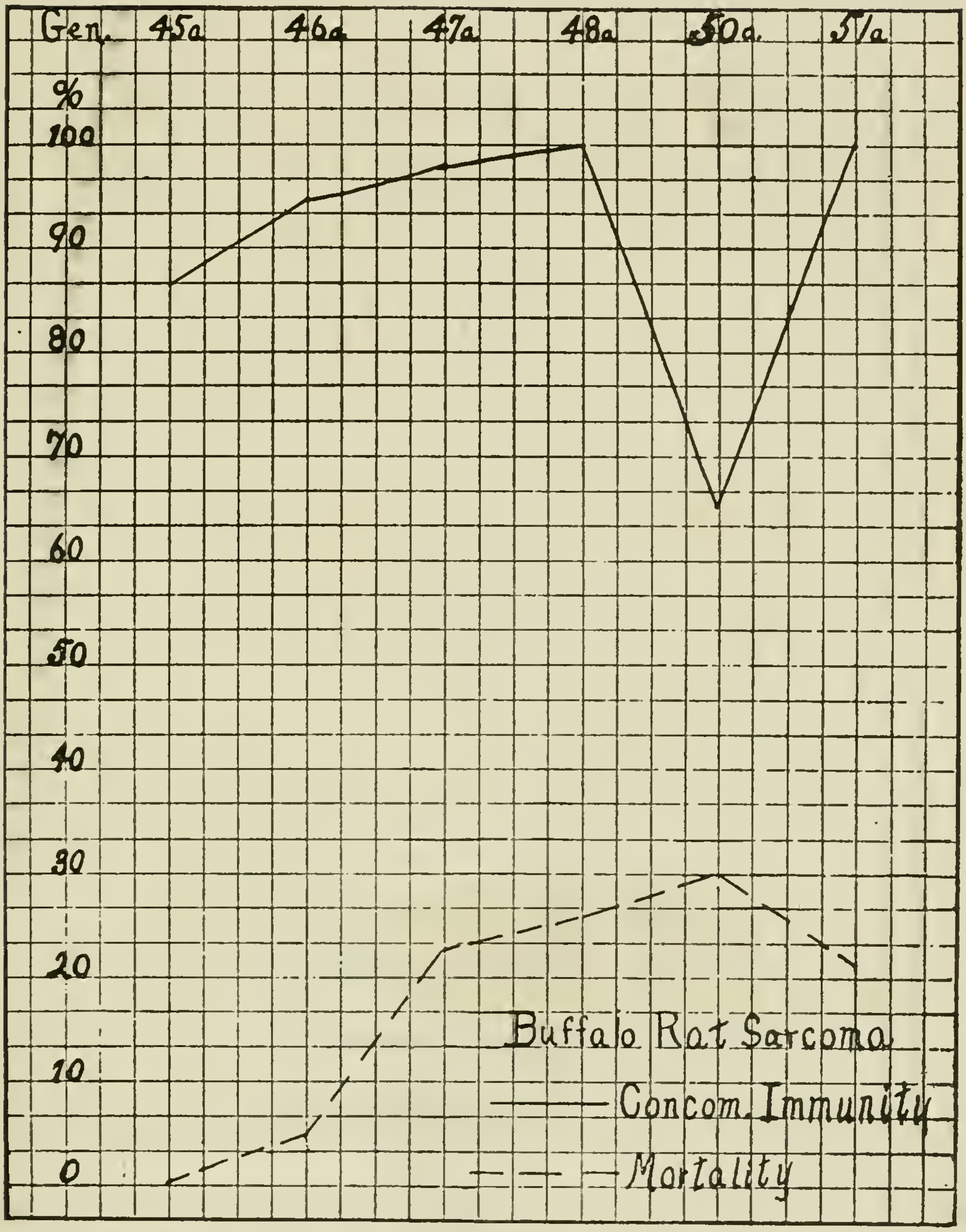

CHART 8. A COMPARISON OF THE PERCENTAGES OF CONCOMITANT IMMUNITY AND MORTALITY WITH THE BUFFALO RAT SARCOMA

so great, the variations observed through seven generations of the Flexner tumor (chart 10) are similar to those noted in the Buffalo tumor, the high point of immunity being 70 per cent, while the low point was 32. With this tumor, also, the concomitant immunity is apparently independent of the induced and the natural immunity (chart 11).

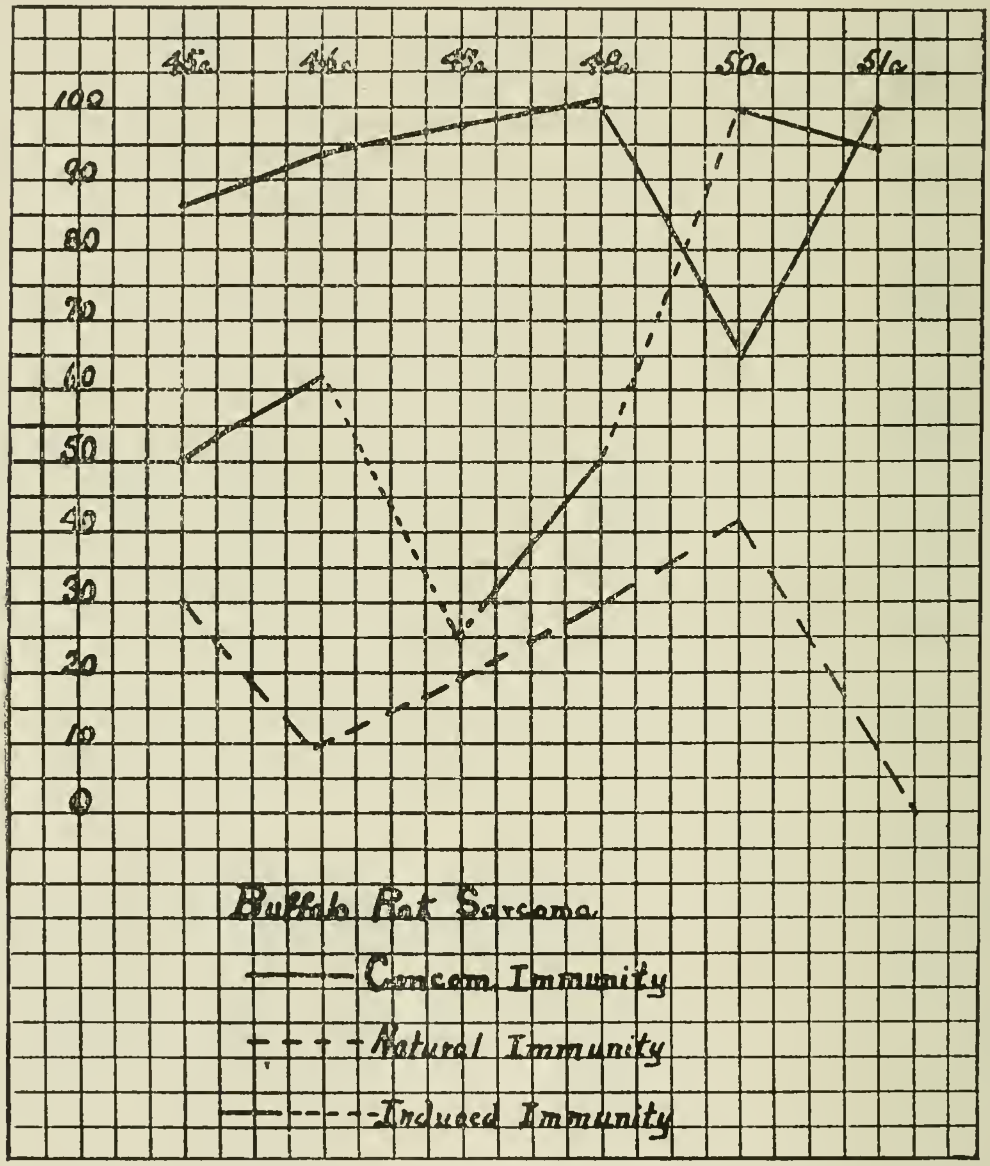

Chart 9. A Comparison of the Variations in Percentages of Concomitant, Induced, and Natural Immunity in the Same Generation of the Buffalo Rat Sarcoma

To obviate the factor of familial or racial tendencies, a factor which is known to influence not only the percentage of takes in transplanted tumors, but also, as we have shown, the percentage of induced immunity, a pure strain of mice was used for the mouse carcinoma 63, both tumor strain and animals being of English stock. Through eight generations of this tumor (chart 12) even more marked and more frequent fluctuations occurred than with the previous two strains, the low being 21 and the

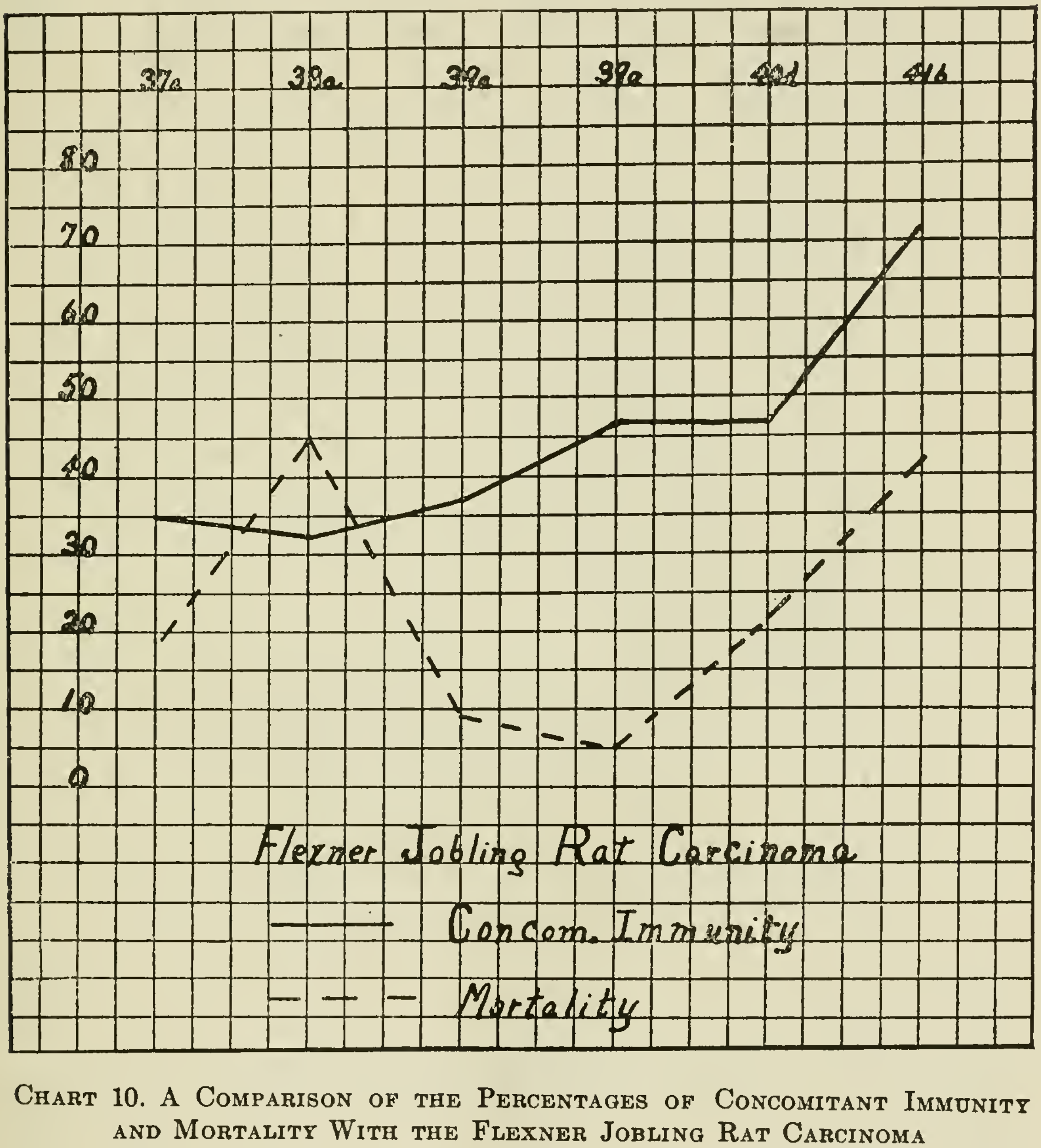

CHART 10. A COMPARISON OF THE PERCENTAGES OF CONCOMITANT IMMUNITY AND MORTALITY WITH THE FLEXNER JOBLING RAT CARCINOMA

high being 60 per cent. As with the other strains, no relationship can be established between the variation in induced, concomitant, and natural immunity (chart 13).

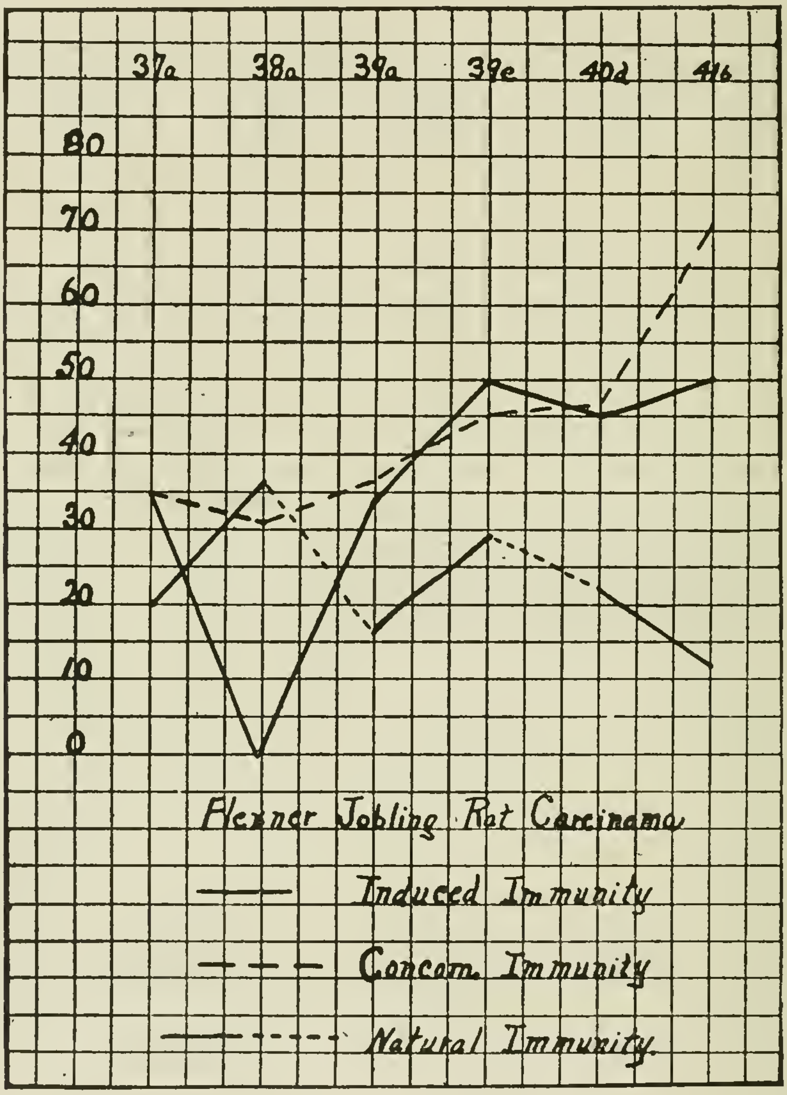

Chart 11. A Comparison of the Variations in Percentages of Concomitant, Induced, and Natural Immunity in the Same Generation of the Flexner Jobling Rat Carcinoma

In charts 8, 10, and 12, the mortality curves and the concomitant immunity curves for each of the three tumor strains are presented. It is evident that there is no relation between the health of the animals, as indicated by the mortality curve, and the percentage of concomitant immunity.

From the experiments which have been described in the previous paragraphs, it is evident that fluctuations in concomitant immunity frequently occur with both epithelial and connective tissue neoplasms in mice and rats. It will be recalled that analagous variations in induced immunity were found to be due to differences in the host strain. This, however, is not true of concomitant immunity. Neither can the variations be connected with similar fluctuations in either induced or natural immunity, apparently somewhat different forces being operative in each instance. Variations in technic have been eliminated by following the standard laid down in an earlier paragraph. By exclusion, therefore, the vacillations must be due to differences in the tumor itself. If, as Bashford has suggested in explaining

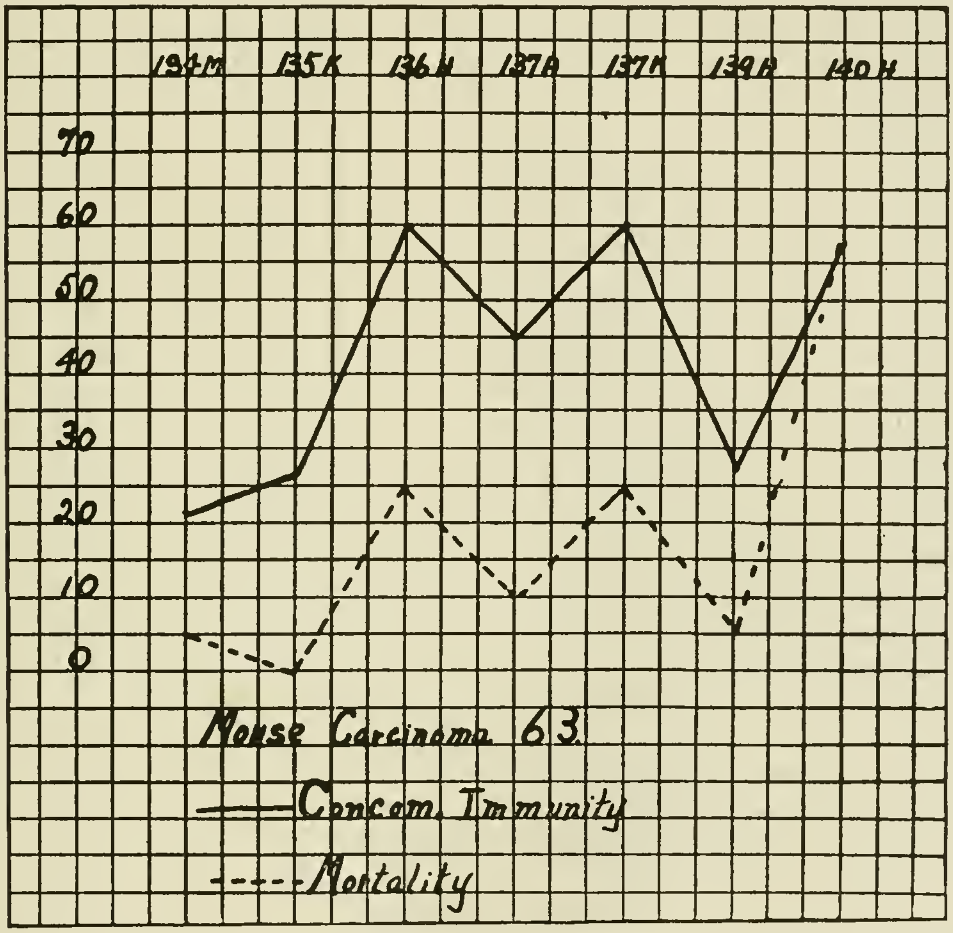

Chart 12. A Comparison of the Percentages of Concomitant Immunity and Mortality with Tumor 63

another phase of tumor biology, these variations are due to fluctuations in the growth energy of the tumor, then growth energy must be measured not by the infectivity of a tumor, as indicated by the number of takes, but by the rapidity of growth of a single given tumor. Though it is probably impossible to establish a standard for size or weight of a given tumor strain after a fixed period of growth, it appears from our experiments that concomitant immunity occurred most often when the individual

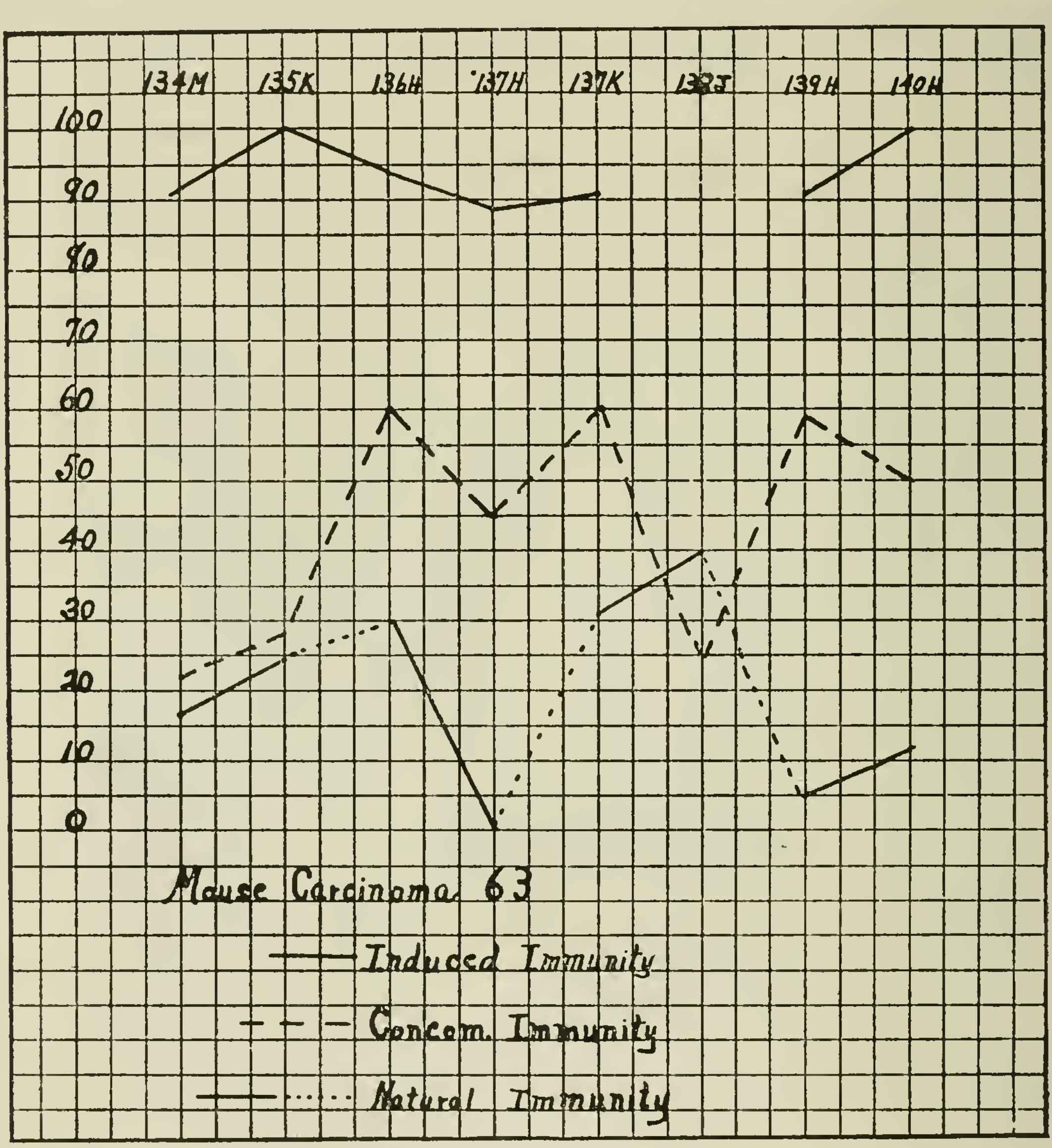

Chart 13. A Comparison of the Variations in Percentages of Concomitant, Induced, and Natural Immunity in the Same Generation of Tumor 63

tumor grew slowly but steadily, and least often when the tumor grew rapidly. Whatever the cause of the variations noted, their presence is proof of the inconstancy of tumors as immunizing agents.

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A BASAL-CELL EPITHELIOMA OF THE RAT

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From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director

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The tumor to be described arose spontaneously in a young male rat, about five months old, weighing 60 grams. When first observed, it appeared as a flattened oval mass, about 2.5 by 1.5 cm. in diameter, occupying the skin of the left thoracic region, and resembled an epithelioma such as occurs frequently on the human face. At the center the tumor was ulcerated, with an uneven granular surface. The margins of skin about this were nodular, raised, thickened, and slightly undermined, and of a reddened shiny appearance which shaded rather abruptly into the normal tissues. The consistency of the mass was distinctly firm, and it was freely movable on the deeper parts.

The tumor was removed by operation, and the major part of it was inoculated into 204 rats and, in two places, into its bearer. Serial paraffin sections which were made of the remaining tissue showed the following histological characteristics:

The tumor was in direct connection with the skin (fig. 1) and extended into the subcutaneous tissue but not into the muscle; it was composed of rounded and irregular alveolar masses of cells having the same staining and morphological characteristics as the basal cells of the epidermis and skin appendages (fig. 2). There were all gradations from typical basal cells to those of a spindle shape. The cells were of small size, with a deeply staining nucleus and relatively small amounts of protoplasm giving the alveoli a very dark appearance. In places these cells were in strands and whorls resembling a spindle-cell sarcoma (fig. 3). Here and there, in the center of cellular areas, were glandular (fig. 4) or cystic structures filled with cell débris and lined by squamous cells in layers; there was, however, no production of keratin. The overlying skin was not abnormal except at one point where the strands of tumor cells and the basal layer of the epidermis were continuous. Staining with the Bielschowski method showed no intercellular collagen fibrils.

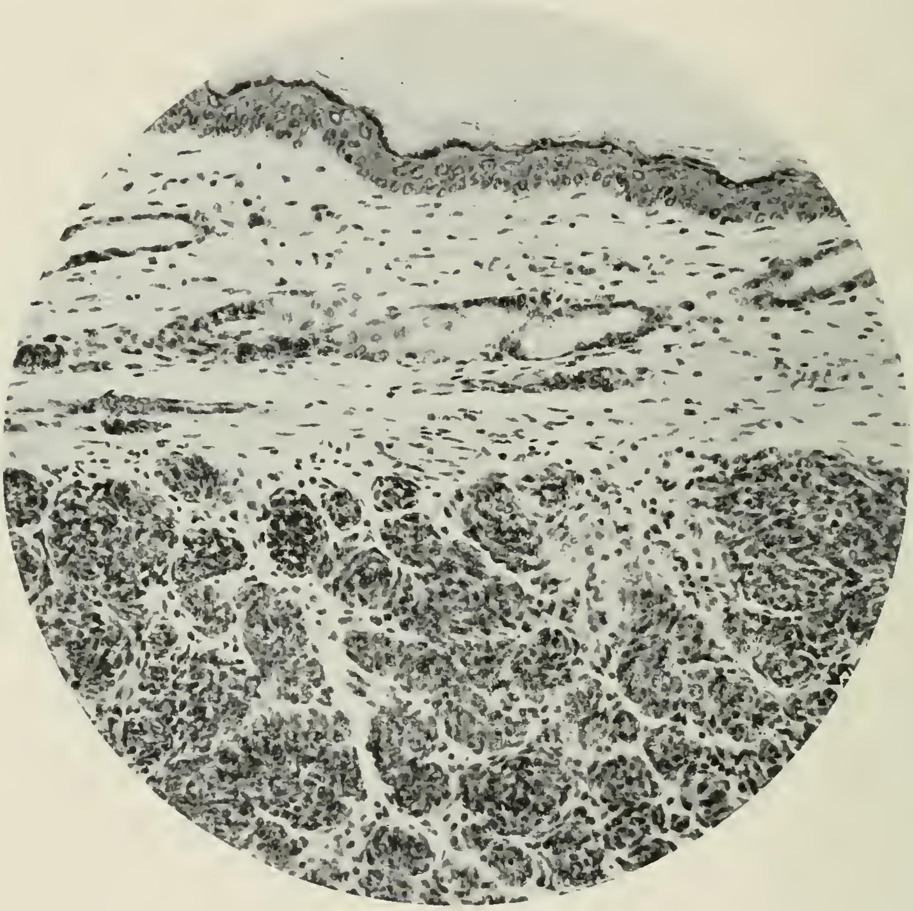

FIG. 1. Basal-cell epithelioma of rat (primary tumor). × 200

The interalveolar connective tissue showed marked proliferation and in places resembled sarcoma. As may be seen in the photomicrographs there was an unusual amount of lymphocytic infiltration about the tumor alveoli.

Seven weeks after inoculation, a few nodules were palpable in the 204 rats, and after nine weeks eighteen nodules in all were present. These increased in size with extreme slowness and six of them reached dimensions sufficient for transplantation into a second generation. The largest was about 1 by 1.5 cm. and required 240 days for its growth.

Transplantations into a second generation were as follows:

1. Tumor 0.9 by 1 cm. 61 days post inoculation into 108 rats.
2. Tumor 0.8 by 1 cm. 62 days post inoculation into 120 rats.

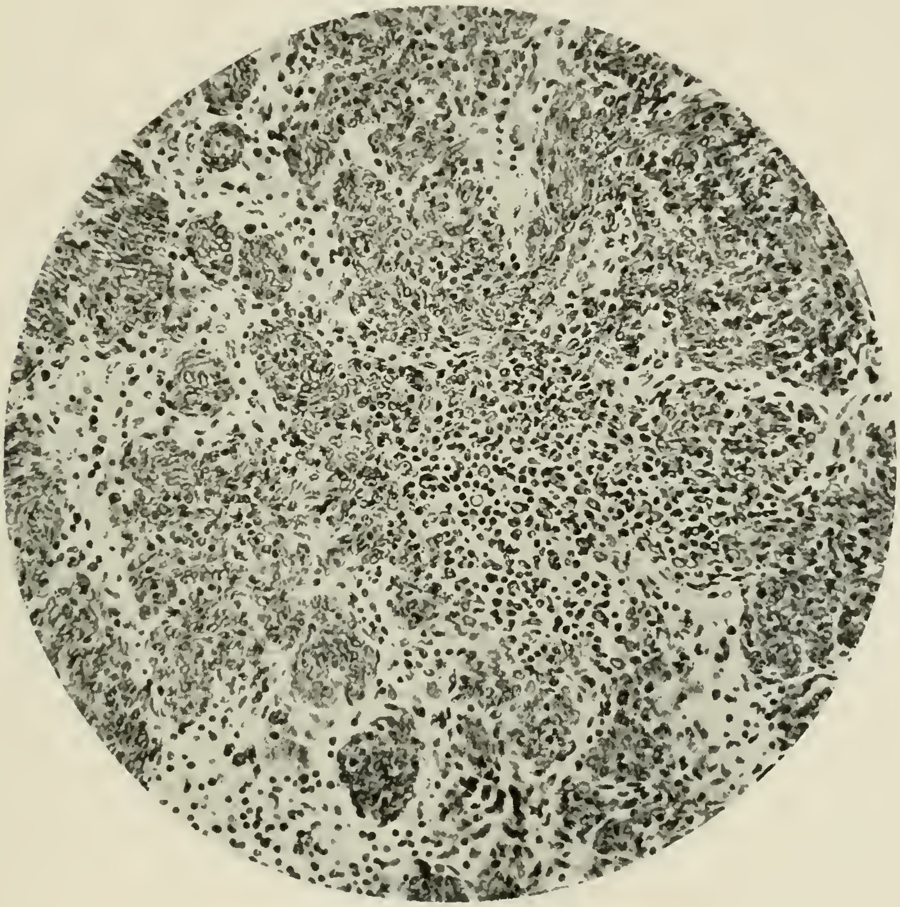

FIG. 2. Basal-cell epithelioma of rat (primary tumor). × 200

3. Tumor (transplant into original tumor-bearing animal) 1 by 1.3 cm. in diameter 111 days after inoculation into 48 rats.
4. Tumor 1 by 1.3 cm. 119 days after inoculation into 54 rats.
5. Tumor 1.2 by 1.3 cm. 135 days after inoculation into 60 rats.
6. Tumor 1 by 1.5 cm. 240 days after inoculation into 68 rats.

In the first five series of the second generation, comprising 390 animals, several minute nodules appeared, but these failed to grow. A difficulty was experienced in the high mortality of the animals from intercurrent disease. In the sixth series of the second generation one reached a size suitable for transplantation. This was transplanted into 48 rats, but failed to grow. One fragment was inoculated into the original animal and grew rather rapidly to a size 1 by 1 cm., when unfortunately the animal died. The histology of this tumor was peculiar. While the characteristic basal cells occurred and the arrangement was typical in places of the spontaneous tumor, elsewhere there was an adenomatous arrangement and in one part a solidly alveolar type of tumor was found with dense masses of cells closely investing blood capillaries.

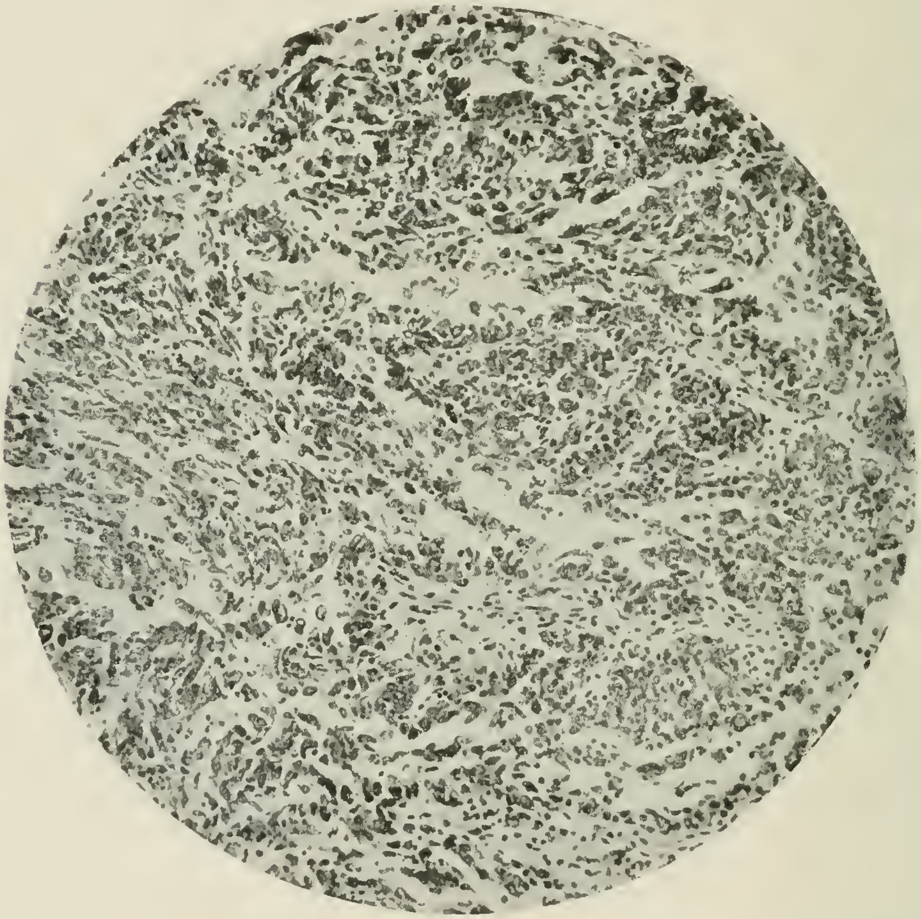

Fig. 3. Basal-cell epithelioma of rat (primary tumor), showing spindle-shaped epithelium. × 200

Microscopical sections of all tumors removed for transplantation showed the same general morphological characteristics as the original growth. Further propagation of the tumor, owing to failure of the grafts, proved impossible and the tumor is now extinct.

Very interesting has been the observation of the grafts into the original animal, which lived for 377 days after the original tumor was removed. Into this animal two transplants from the spontaneous tumor were made, one into the right axilla and a second into the right groin. Growth in these did not start for nine weeks, or later than the appearance of the nodules in some of the other rats of the first generation. This is contrary to the usual experience in autotransplantation. As soon as growth had begun, however, both tumors grew progressively and fairly rapidly, until at 111 days after inoculation the tumor

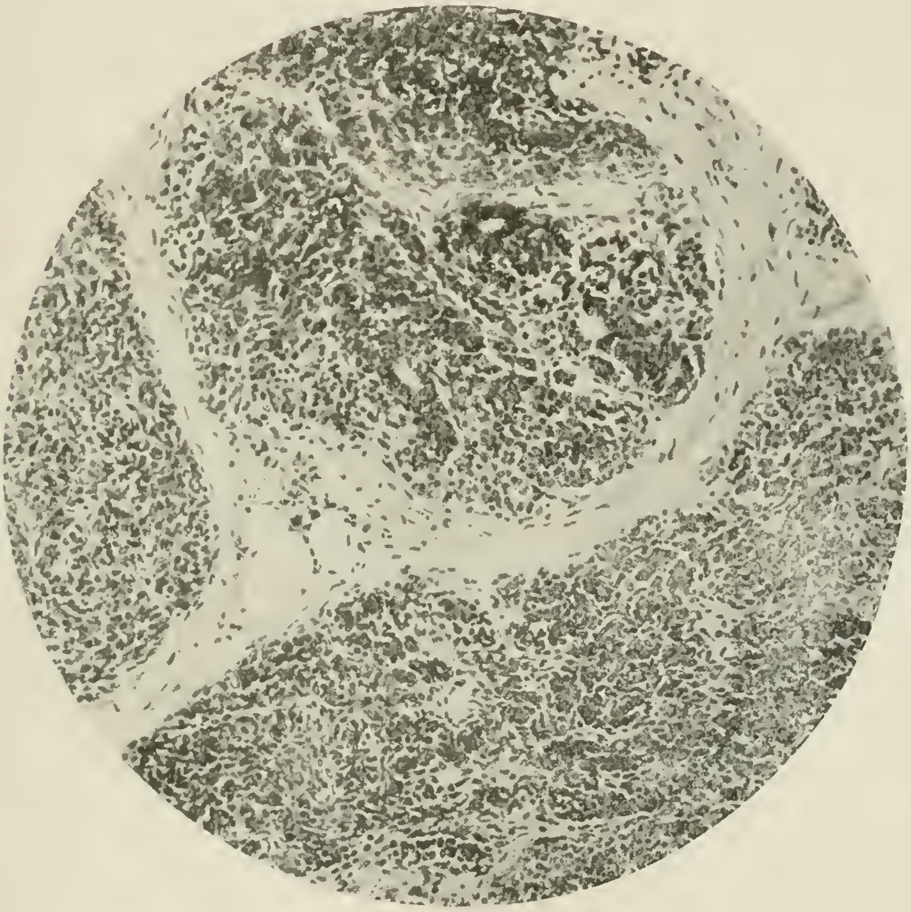

Fig. 4. Basal-cell epithelioma of rat (primary tumor), showing alveolar areas. × 200

in the axilla had attained a size of 1 by 1.3 cm., when it was removed for transplantation. Two fragments of 0.003 gram were inoculated into the left axilla and left groin. These grew to a size of 0.5 by 0.5 cm. each and then completely receded. The tumor in the right groin remained stationary without increase or decrease of size after the removal of the axillary tumor. Three hundred and seventy-seven days after inoculation, the animal died and this tumor was removed for microscopical examination. It showed in places the same histology as the original tumor and elsewhere a peculiar adenomatous arrangement of the cells. It was free from degenerative changes and mitoses were very scarce.

Subsequently, two grafts from a tumor of the second generation were transplanted on right and left sides beneath the skin of the back of the animal in which the tumor originated. Both of these after reaching the size of a split pea completely receded. Failure to grow was not referable to infection of the tumor for it grew in other animals.

If we attempt to analyze these fluctuations of growth energy in the original animal we meet with some very interesting questions. If the primary growth and subsequent recession of the four grafts that underwent resolution be regarded as the development of immunity in the animal, why was not the tumor in the right groin likewise absorbed? Furthermore, the development of any immunity whatsoever to its own growth by a spontaneous tumor animal has been thought not to occur (1) and certainly ordinary immunizing injections of homologous tissue fail completely to cause it. Yet if we do not regard the recession of four grafts in an animal as the development of a species of immunity, how is it to be interpreted? The phenomenon of the recession of one spontaneous tumor while others continue to grow has been observed in rare instances before; thus Haaland (2) records an instance of a tumor in one groin receding in the animal in which it arose while an axillary tumor of the same variety continued to grow. He records also an instance of complete temporary recession of a spontaneous tumor of the vulva. Both these phenomena are, however, conspicuously rare.

A second feature worthy of note is the remarkable length of time (266 days) which the graft in the right groin of the original host remained dormant without increase or decrease of size. This is most unusual. This nicely adjusted balance which permits cancer cells to live so long in a host and yet not perceptibly multiply is more characteristic of the benign tumors, which until quite recently have not been successfully transplanted, or of lymph-node metastases, which in man have been recorded as lying dormant for ten years or more.

Another question which is aroused by the failure of the four grafts in the original animal is, why cells which are native to one animal should first grow in another animal and then on return to the original host fail to grow? These things are contrary to the customary experience in autologous transplantation and merit further research.

This tumor, as far as can be ascertained, is the only basal-cell epithelioma that has been found in an animal. Its sluggish growth rate and ability to lie dormant for long periods is quite analogous to the behavior of the same type of tumor in man.

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A STUDY OF FOUR CASES OF BEGINNING SQUAMOUS-CELL CARCINOMA OF CORNIFYING TYPE

R. C. WHITMAN

From the Denison Memorial Research Laboratories

Received for publication, November 5, 1919

INTRODUCTION

The following pages are the result of a study of cases of very early and actually beginning carcinomata of the skin. In an earlier paper (1), I discussed in some detail von Hansemann's theory of anaplasia, and tried to indicate how naturally and easily that theory develops, in the light of present knowledge of the laws of inheritance, into one which regards the cancer cell as a product of somatic mutation. So simple and direct, indeed, is that development, that the difference is largely the difference between the terminology of thirty years ago and that of today. It follows of course that the present point of view contains little that is new. The anaplastic cell of von Hansemann becomes quite naturally the new race of cells of Hauser; so that I am by no means the first to apply either the concept or the term, mutation, to cancer genesis. But the application to the problem of the remarkable observations of Morgan and his associates gave me, I felt, such added understanding that it seemed worth while publishing it. The present paper attempts to approach the problem from the same viewpoint as the earlier one but to present it in a more objective, less abstract form. Since it is largely based on the same bibliography as the preceding paper, it has seemed unnecessary to repeat that given there.

The present study embraces seven cases about equally divided between the cornifying and the non-cornifying types. The non-cornifying tumors will be discussed under a distinctive title.

Ribbert (2) bases the distinction between the two forms on the mode of growth.

> In all cases where infiltration occurs by means of continuous elongation of the epithelial papillæ or processes, a cornifying carcinoma is produced, ... but when on the other hand entirely new-formed, independent buds invade the cellular connective tissue, growing from the basal layer of the epidermis or from the lateral aspect of the epithelium of the hair follicles or sebaceous glands, and especially from the lower epithelial layers, which have been converted into light staining, large cells, then a non-cornifying carcinoma usually develops.

I have made use of this criterion as far as it would serve. But as will become evident, it has not always served, since the type of growth does not always coincide with one or the other of these categories. On the whole I have felt that I could more safely rely on the following points.

1. *a.* Cornifying carcinomata, so far as my series is a guide, arise in verrucous keratoses.

b. Non-cornifying carcinomata, on the other hand, arise from areas where there is no verrucous thickening; often indeed, the epidermis is sunk below the level of the normal skin, apparently because of changes in the corium. The cases of doubtful classification in my series are of the first type, *a*, and I am by no means sure that the above furnishes a reliable index.

2. A more satisfactory guide is furnished by the character of the malignant cell. In the non-cornifying tumors, this very quickly settles into its established type, as a small, more or less spindle-shaped cell with deeply staining nucleus and scanty protoplasm; whereas in the cornifying tumor, the cells remain for a longer time at least, with larger, rounder, more vesicular nuclei and more abundant and more opaque protoplasm. Where the formation of pearls is early and abundant, the diagnosis is of course settled at once.

To save space I shall describe here briefly, once for all, certain degenerations which occur quite commonly in the connective tissue, especially in the cornifying cases. These changes are described in detail by Unna (3) as senile degenerations, occurring regularly in the skin of all old people (50 years or more), especially of the face. The following is a much shortened description from Unna.

1. *Elacin.* Constant in the middle and deeper layers of the cutis; single and in bundles, broad, cylindrical, and wavy fibers which look deceptively like ordinary elastic fibers, but stain with basic dyes. The same fibers stain with specific elastic fiber stains, though somewhat less intensely than normal.

2. *"Blue areas."* Immediately under the basement membrane in and around the areas in which elastic tissue is thickened and apparently increased, collections of basophilic collagen fibers which swell, lose their sharpness of contour, and break up into clumps and granules of varying size.

Unna's collacin and collastin degenerations I have not seen, probably because I have not looked for them in properly prepared sections. The blue areas in my preparations stain more or less deeply with hematoxylin, depending on the length of time the sections are left in the stain and the degree of subsequent differentiation (in acid alcohol). If the latter is pushed far enough the hematoxylin is entirely removed, but the areas resist subsequent staining with eosin. The same areas stain evenly with elastic fiber stains (I used Verhoeff's). I assume that these areas also contain collacin, which does not appear in the exact form described by Unna, because Verhoeff's stain is less precise than the orcein method employed by him. The elacin degeneration and the "blue areas" are mentioned only because they constitute a striking feature of several of the cases. The "blue areas" in particular seem to be identical with the so-called "precancerous condition of the connective tissue" seen, sometimes in conjunction with myxomatous degeneration, about the hair follicles in carcinoma of the skin, or about groups of gland acini in the breast. It is apparent that their presence is merely incidental. Similarly with regard to other and more delicate senile changes in the skin, such as thinning of the epidermis, with local thickening and pigmentation of the horny layer, shortening and disappearance of the epithelial papillae, changes in the hair follicles, depressions or dimples in the surface of the skin, etc. All these are incidental to old age and will be mentioned, if at all, only incidentally. Of greater interest to the present discussion are the foci of leucocytic infiltration often found in the cutis, according to Neumann (4). Their frequent presence in the senile skin argues strongly against their having any such significant relation to cancer genesis as is assigned to them by Ribbert.

One more word of introductory explanation seems necessary to guard against a possible charge, through misunderstanding, of serious and obvious blundering on my part. I shall have frequent occasion to describe varying stages of malignant growth as occurring side by side in the same area. Usually the more advanced stage is found near the center of the area involved, with younger growths scattered about in the neighborhood. These younger growths I do not consider to be metatases, for what, I trust, will be regarded as obvious reasons. For equally obvious reasons they are not to be regarded as indicating a spreading infection, nor as appositional growth, i.e., secondary "infection" and conversion into carcinoma cells of neighboring normal epithelium by the cells of the earlier and more advanced growth. We must assume, I think, that mutative changes can occur only during the process of mitosis. This being the case, the presence in a given area of those unknown conditions favorable to cancerous mutation is not enough, taken alone, to produce a cancer. Their effective functioning must wait upon the occurrence of mitosis. Doubtless also many mitoses occur in the area, without falling victim to the disturbing influence; and since it is hardly conceivable that any considerable number of mitoses should occur simultaneously throughout the area involved, and since, even if this should happen, it by no means follows that the rate of growth in all the cancers thus started will be alike, nothing is more naturally to be expected than that any area of multicentric growth will exhibit many independent growths of various ages, or at least, stages of progress.

I shall describe the cases in the order of their stage of development, beginning with the youngest.

CASE 1. CORNIFYING CARCINOMA ARISING MULTICENTRICALLY FROM BASAL AND PRICKLE CELL LAYERS OF EPIDERMIS AND HAIR FOLLICLE

This was one of two senile warts removed in 1909 by Dr. C. B. Lyman from the back of a man about seventy years old. Over an area of about 6 mm. in diameter there is a moderate rough keratotic thickening rising 1 to 2 mm. above the level of the normal skin (fig. 1). In the region surrounding this elevation, areas of the germinal layer of varying size contain a finely granular dark pigment. These cease a short distance from the edge of the verruca. Under the basement membrane, and extending the entire length of the section, except immediately below the verruca proper, is a succession of irregularly round or oval blue areas (fig. 1, *a*). The basement membrane is a delicate layer of collagen fibrils, varying somewhat in thickness. Even at the extreme ends of the sections, it exhibits a tendency to become hyalin and lose its nuclei. As the center is approached this tendency increases, the fibrils swell and break up into coarse lumps which still retain their normal color reaction to eosin; the nuclei disappear almost completely and the whole structure is finally replaced by a hyalin vacuolated mass suggesting a coagulum. But the process is not continuous and areas of more normal appearing basement membrane occur here and there. At the edge of the verruca where budding of the epithelium is beginning, the epithelial cells of the basal layer abut on a very narrow open cleft, beneath which is a delicate stroma of degenerated, partly disintegrated collagen fibers (fig. 2, *1*). There is a scanty small round-cell infiltration about the blue areas and between the latter and the epidermis; also and more abundant about the hair follicles, sebaceous glands, and the capillaries supplying these.

The epidermis beneath the parakeratotic area is thickened and exhibits many irregularly shaped buds extending in various directions into the corium. Some of these are found at some little depth in the corium, cut in cross section and apparently free and detached from the epithelium. The corium here is only slightly infiltrated with small round cells, and this infiltration is practically confined to the zone of connective tissue immediately surrounding the epithelial buds above mentioned. I shall describe some of these buds in greater detail.

The area shown in figure 2 is a higher magnification of the area shown at the left in figure 1, marked *b*. The bud at *A* consists entirely of cells resembling somewhat those of a non-cornifying carcinoma, but with more abundant and pink staining protoplasm. The nuclei are round or oval, dark, and not regularly arranged with their long axes at right angles to the plane of the basement membrane, which as already noted, is partly deficient. The oblique line marks approximately the line of separation between visibly altered cells, and those apparently still normal. The still intact basal layer of cells can be seen extending across the base of the bud about one half of the distance from the left to the right, where the line *A* terminates. The bud itself seems to arise from that portion of the basal layer to the right of this point. This represents a very early stage of visible change in the cells. Indeed taken by itself, it could not of course be recognized as a step in the malignant process.

The larger bud at *B* exhibits a somewhat later stage. At the point where the basal cells turn inward over the bud (*1*), the basement membrane suddenly disappears. From this point leftward the basal cells change progressively in character. Here the crooked line drawn through the bud marks the approximate line of demarcation between the proliferating basal cells above and to the left (in the photograph) and the prickle cells below and to the right. The former are distinctly plumper, deeper staining, and more irregularly arranged than normally. They show a distinct tendency to group themselves in spherical masses or lobules, similar to the groups sometimes seen in mucous membranes at the beginning of papilla formation. At *3*, they are beginning to send delicate protoplasmic bridges suggestive of pseudopodia, into the degenerated, partly disintegrated connective tissue. Significant changes however are not confined to the basal cells. The prickle cells are large, and less flattened and their nuclei darker and less spindle-shaped than is proper to spindle cells of the corresponding level of normal epidermis. At three points (marked *2*) there are small groups of darker cells concentrically arranged, corresponding to an early stage of pearl formation, or to cell groups isolated in situ and growing independently. Considering that this particular bud is at the extreme edge of the verruca, and the absence of pearls in the verruca itself, the formation of pearls here is not without significance. More striking evidence of involvement of the prickle-cell layer will be cited shortly.

Figure 3 is a higher magnification of the point marked *c* in figure 1. It shows part of a cancer bud of considerable size. This is connected with the epidermis covering the verruca above by a strand of elongated prickle cells which passes through the basal layer and can be followed almost to the horny layer (*a*). The cells composing it are sharply differentiated from their neighbors within the prickle-cell layer by their lighter color, more elongated shape, and sharp difference in the angle at which their long axes are placed. The whole picture is curiously suggestive of a crowd streaming through narrow doors. By no possibility can it be interpreted as due to secondary adhesion of malignant to normal epithelium, or otherwise than as indicating the origin of the cancerous growth from the prickle-cell layer.

Figure 4 is a higher magnification of the area marked *d* in figure 1. The dark mass of epithelium in the center is a hair follicle. At either side (probably indeed encircling it in the tissue), are unmistakable cancer buds springing from the point of juncture of the hair follicle with the epidermis and extending slightly further down the length of the follicle on the left than on the right. It is reproduced partly because it seems to offer further incontrovertible evidence of the share taken by the prickle cells in the production of the cancer (observe especially the large deep staining unmistakably mutated (anaplastic) prickle cells at the base of the bud on the left); and partly because of the relation stated by Mallory (5) to exist between the hair follicles and non-cornifying carcinoma. This carcinoma, which belongs, I think, to the cornifying type, arises from the hair follicles as well as from the epidermis. Compare Ribbert (2, p. 155 and fig. 119) where a like origin of a non-cornifying carcinoma is described. Near the center of the picture, and just to the left of the hair follicle is a group of carcinoma cells which was probably continuous with the downgrowth at the right of the hair follicle.

At several points (fig. 1, *e* and *f*), more advanced stages of growth can be seen—fairly deep-seated buds unmistakably malignant in character, some with large pearls in various stages of formation. In spite of the fact that the cell buds do not grow straight into the tissue, but send out many secondary buds (fig. 3, for example) very early, and in spite of the fact that the cells of the rather large group shown in the center of figure 4 are rather small and deep staining, i.e., of the non-cornifying type, I am convinced that this case should be classified as cornifying. Possibly it represents one of the mixed type described by Krompecher.

The outstanding features of this case are:

1. The striking absence of inflammatory reaction except in the sharply circumscribed zone immediately surrounding some of the older and more advanced buds, and about certain hair follicles and sebaceous glands.
2. The very early stage of the growth in many areas. Many of these, taken by themselves, could not be regarded as indicating malignancy. But the location of these growths inside the margin of the verruca excludes ordinary inflammatory proliferation of the epithelium with the single possible exception of the small bud first described and shown in figure 2.
3. The multicentric growth, involving both the basal cells and the middle layers of the epidermis.
4. Origin from hair follicles as well as from epidermis.

CASE 2. CORNIFYING CARCINOMA ARISING DIFFUSELY FROM GERMINAL LAYER OF EPIDERMIS

This is a keratotic wart (fig. 5) removed by Dr. O. M. Gilbert from the scalp of a woman about fifty years old. It is approximately 5 mm. in diameter and rises abruptly above the surface of the surrounding skin. In general it resembles case 1 closely. The striking points of difference may be summarized as follows:

1. Blue areas occur as in the preceding case, and in addition small areas may be found in the markedly inflamed corium underlying the verruca.

2. Elacin degeneration of the elastic fibers is present to a marked degree among the elastic fibers in the deeper layers of the cutis.

3. The inflammatory reaction is slight beyond the margin of the verruca as in case 1, but beneath the verruca is much more intense and widespread. In the area surrounding the verruca the blood- and lymph-vessels are distended, their endothelium is somewhat plumper than normal, and the vessels are accompanied by a scanty accumulation of small round cells. Under the verruca the blood capillaries contain an occasional hyalin thrombus and are frequently filled with leucocytes, and the surrounding tissue is quite densely infiltrated with wandering cells, among which polymorphonuclear cells, while not the predominating type, constitute a striking feature. The inflammation extends almost to the depth of the lower terminations of the sweat glands.

4. *The epidermal cells.* The cells of the granular layer are here and there, especially near the outer border of the verruca, infiltrated with leucocytes, chiefly polymorphonuclear cells. The cells of the prickle layer are somewhat large and plump, i.e., less flattened than normal. At one point only (fig. 5, *a*) I have found a bud of such size that an offhand diagnosis of carcinoma would be possible. It extends deep into the corium, in the form of an inverted Y, and consists of rather large spindle-shaped cells, whose long axis is directed for the most part inward (or downward); the nuclei are rather large, very pale, vesicular, round, oval, or spindle-shaped, with one or two large, deep staining nucleoli (fig. 6). Protoplasmic bridges between the cells can be made out in a few places. The contour of the bud is somewhat irregular. Over part of its extent it is bounded by a somewhat diffuse pseudo-basal layer consisting of cells like the rest except that they stain more deeply. The tips of the bud are somewhat infiltrated by round cells and become lost in the fairly dense inflammatory infiltration about them.

The precise center of origin of the process from the overlying epithelium is not in the sections. It lay, I take it, too nearly parallel with the plane of the section to show, or else it was discarded in some of the sections torn in cutting; but a connection, clearly not secondary, can be seen with the tip of the overlying epithelial papilla, where the basal cells are everted over the beginning of the bud and cover its base for a certain distance (fig. 6, *a*). The prickle cells continuous with the process inside the everted basal-cell layer show no abnormality in size, shape, or staining reaction, and I therefore infer are taking no essential part in the growth.

There is no pearl formation anywhere but the type of cell in this bud settles the diagnosis as cornifying carcinoma.

At many places the basal-cell layer over considerable stretches shows a marked heaping up. The earliest stages of this change cannot, of course, be recognized with certainty. Apparently the basal cells first become somewhat larger and darker, so that they stand out with more and more distinctness and tend to lie flat side to the basement membrane (fig. 7). Later they increase to many layers in thickness, become more and more disorderly in their arrangement and tend to separate from one another (fig. 8). In this condition they are readily distinguished from the familiar artefact, due to oblique cutting. Apparently at any stage actual budding may occur (figs. 8 and 9). In some cases at least, such budding takes place by the growth inward and laterally of a small group of basal cells, in such a way as to carry the basal cells on either side with it for a certain distance (fig. 9). In this way it produces perhaps the inverted basal-cell layer described above.

Figure 10 shows early growth from the upper portion of a hair follicle. The only question which can arise, I think, is whether the growth here shown is due to changes in the follicular epithelium proper or to extensions along the follicle of growth arising in the neighboring epidermis. The absence of any line of demarcation between normal and proliferating cells settles the question in favor of the former interpretation.

Figure 11 is the epidermal layer from a large verruca, for comparison. It will be observed that here too a fairly marked inflammation is present. The orderly disposition of the cells forms a marked contrast with the earlier figures. I have frankly accepted, as belonging to the cancerous process, changes in the cells which, taken by themselves, would not warrant that diagnosis. This is in accordance with my conviction, as explained in greater detail later, that a disease (typhoid, for example), begins at the moment the disease process is set in motion. In the present case stages of the disease are present which are clearly recognizable as such: there are other, and earlier stages which, standing alone, could not be so recognized. But the relation of the latter to the former can be traced. It is clear that they belong together.

The case is, then, one of cornifying carcinoma beginning rather diffusely in the basal layer of epidermis and hair follicles and without appreciable involvement of the more superficial layers.

CASE 3. CORNIFYING CARCINOMA ARISING DIFFUSELY FROM THE BASAL-CELL LAYER ONLY OF A VERRUCA

This is a verruca measuring 4 mm. in diameter, removed from the helix of the ear of a man, fifty-five years old, by Dr. C. E. Giffin. Its top is covered by a thick, rough, laminated mass of keratinized epithelium which is attached at its base to epithelium covering a fairly deep and sharp depression in the surface of the skin (fig. 12). The lower (inner) level of the epithelium over this depression roughly corresponds to the level of the corium containing the papillae of the hair follicles of the surrounding skin. But the skin of this region rises slightly to meet the sides of the verruca, and the epithelial papillae become gradually longer and thicker in the same direction. At the edges (or ends) the basal layer is pigmented in spots, which disappear toward the center. At the edges also the epithelium rests upon a sharply defined, firm basement membrane of connective tissue, whose fibers run parallel to the surface of the epithelium which they support. Toward the center again these fibers swell, lose many of their nuclei, become hyalin, then break up into lumps and coarse granules, and, finally, at or near the edge of the verruca disappear here and there, leaving a narrow cleft containing granular detritus. Across this cleft extend (in some sections) delicate epithelial processes which increase in size and number, as one passes inward along the first large epithelial papillae belonging to the verruca, as in case 1 suggesting pseudopodia.

Under the basement membrane the collagen fibers of the cutis are converted into a dirty blue staining, granular and lumpy mass, almost entirely devoid of nuclei. The line of incision for the operation passes through this level, so that the condition of the deeper layers of the cutis cannot be determined. The region containing blue areas ceases abruptly at the edge of the verucca proper, the greatly elongated epithelial papillæ of which extend into the cutis far beyond the level at which the degeneration is found on either side.

One of the most striking peculiarities of the case is the almost complete absence of wandering cells. A very few minute foci may be found, partly near the edges of the sections, in connection with the blue areas, partly in the region of the verruca, gathered about buds of proliferating epithelium.

Cursory examination of the sections creates the impression that the basal layer underlying the verruca proper has disappeared, and that proliferation is taking place from the prickle-cell layer only, by means of long branching and anastomosing streamers of cells, extending into the corium. More careful study shows that the earliest stages of growth take place by a process similar to, but not identical with, the bleb-like proliferation which characterizes case 4. Here however, the change obviously involves the basal cells primarily, rather than the prickle cells as in case 4. In the present case the basal cells, as the first visible step in the process, become larger and more deeply staining than normal, become separated from the overlying prickle-cell layer, and bulge inward toward the connective tissue. In this condition they are often still arranged regularly side by side, and being taller than normal, they closely resemble a columnar-cell mucous membrane (fig. 13). At the next stage the basal layer is found thrown into folds whose tips, at first pointing inward, fold and branch in various directions in the underlying connective tissue (fig. 14), and grow into the capillaries and lymph-vessels. Here they become arranged on the walls about a central lumen, replacing the endothelium. In some of these, structures resembling red cells, really spherical masses of keratin, may be found. Thus here and there the growth presents a resemblance not too remote if allowance be made for the fact of malignancy, to the so-called parotid mixed tumor (fig. 15). The cells now branching and anastomosing along the paths furnished by the lymph-channels, the picture is finally presented of a tangled mass of branching lymph or blood channels formed of epithelial cells. The cells forming these channels undergo keratinization, and the lumen thus becomes filled with a material resembling hyalin collagen, but which is clearly of epithelial origin. This change increases to the abundant formation of large and characteristic pearls. In some of the latter protoplastic bridges still exist between the cells.

Finally, the cells lining these gland-like channels proliferate, both inward toward the lumen and outward toward the surrounding connective tissue. This converts the gland-like structure into solid cords of cells which still anastomose with one another but gradually expand and obliterate the intervening connective tissue.

In the meantime the cells of the altered basal layer have proliferated outward (toward the prickle-cell layer), as well as inward (toward the corium) (fig. 13). Apparently the cells thus formed apply themselves closely to the pre-existing prickle-cell layer and become indistinguishable from these. It thus becomes practically impossible to determine what share, if any, the prickle-cell layer has contributed to the formation of the cancer. But it is, I think, clear that the presence of keratin and protoplasmic bridges in the tumor cells, as above described, cannot be taken as in the least proving the origin of the tumor from the prickle-cell layer, as is evident also from the genetic relationship of the two layers.

As the operation did not remove quite all of the growth, it is impossible to determine just how far its roots extend. It is perhaps the most advanced of the series, and in spite of the fact that its processes do not grow straight downward without branching, and in spite of the small size of its cells, the abundant pearl formation places it indisputably in the cornifying group. Here and there small aggregates of cells of the non-cornifying type are encountered. Several of these, traced through the series, proved to be parts of hair follicles, and I am quite sure that none of them represent neoplastic growth.

CASE 4. CORNIFYING CARCINOMA ARISING DIFFUSELY FROM PRICKLE-CELL LAYER ONLY

A verruca removed by Dr. C. E. Giffin from the face of a patient about sixty-seven years old. The keratotic area is 5 mm. in diameter and arises some 3 mm. above the surface of the surrounding epithelium.

In section, changes similar to those already described are found in the basement membrane. Just under the basement membrane are a few scattered foci of leucocytic infiltration among which a rather large number of eosinophiles form a striking feature. Still deeper is a broad zone in which the collagen fibers have undergone the basophilic change already described. This zone extends almost unbrokenly beneath the base of the verruca from side to side, answering perhaps to the fact that the base of the latter is less depressed below the level of the surrounding epidermis than in the other cases. Beneath the blue areas are small collections of elastic fibers which have undergone elacin degeneration, but this change is less intense and less widespread than in the other cases.

Turning to the epithelium, we encounter what at first sight appears to be two separate and distinct modes of cancerous growth existing side by side. First, the epithelium in the right half of figure 16 is increased to five or six times its normal thickness. But this thickness is due only in part to a diffuse plumping up of the epidermal layer. Most of it is apparently due to the elongation of papillae which also spread laterally and unite with one another more or less completely, along their edges or lateral aspects. This will be more evident from figure 17 which is from the same area but at a different level, and in somewhat higher magnification. Here there is no granular layer. The outermost cells of the prickle-cell layer are very pale and apparently hydropic, as indicated by the many large clear spaces separated by mere shreds of cells. Many nuclei are represented by shadows only, and many seem to have disappeared completely. At a certain level the cells show a distinct tendency to change the direction of their long axes, so as to lie at right angles to the surface (*A*). From this point inward they grow somewhat irregularly, so that sometimes a papilla is narrower at its base than at its tip (*B*), due to pressure at its sides, and papillae apparently fuse when this pressure becomes great enough (*C*). As the cells progress inward they become also more and more atypical. At the points marked *D* this departure from type is well marked and the growth is distinctly infiltrative.

The surprising thing is that all this takes place within a basal layer which is well preserved, and for the most part normally arranged. This basal layer rests upon a basement membrane whose delicate fibrils are widely separated by fluids, and in part are no longer in direct contact with the basal cells. Practically nowhere is the membrane completely lacking.

In other sections from this same region, unmistakable malignant budding is in progress. The character of this budding will be described in detail in connection with the discussion of the left half of the field shown in figure 16, and is therefore omitted here.

Mitotic figures are abundant in the zone of thickened epidermis above described, both in the lower layers of prickle cells (fig. 18) and in the definitely cancerous buds above mentioned. Many of these are markedly hypochromatic in von Hansemann's sense. In one field, for example, I found two monasters in a non-malignant area, one of which contains something like thirteen chromosomes, the other apparently nine; near these, lying free in the cavity of a small gland-like malignant bud, a degenerating cell containing five. Mitoses cannot of course be shown entire in photographs since the lens lacks depth of focus to include the whole figure. I am omitting a detailed description of this phase of the question at this time, partly because it would extend the present paper unduly and partly in the hope that more accurate observation will become possible when I can obtain a binocular microscope.

The apparently second mode of growth is encountered at various levels in the region corresponding to the left half of figure 16, and, at other levels than that shown in the figure, in the region corresponding to the right half of the same figure.

The earliest stage I have been able to identify with any certainty, is shown in figures 19 and 20, from the area just within the edge of the verruca. The whole epidermal layer is thickened, because (aside from increase in the horny layer) of an increase in the prickle cells. This thickening begins rather sharply at the exact edge of the verruca and then increases gradually toward the center. At about the middle of the prickle-cell layer the cells quite abruptly turn their long axes perpendicular to the surface. Here and there small groups of cells, somewhat darker than their neighbors, are found lying free in small pockets in the epidermis. The outermost row of cells of these groups is closely applied to the neighboring prickle cells and from some of them extensions of proliferating cells can be traced downward toward the basal layer. At the same time the cells of the basal layer become taller than normal and though still quite regularly disposed, bulge inward so as to present an irregular, wavy, lobulated outline. The basement membrane has entirely disappeared, its place being taken by a rather broad cleft partly filled by a vacuolated hyalin coagulum. In other areas vacuoles, really minute blebs, appear among the prickle cells just above the basal layer. These, enlarging, coalesce (fig. 21). It is perhaps significant that although many mitotic figures, some atypical, are to be found as already mentioned among the prickle cells, I have not found a single one in the basal layer.

Later stages are seen in low magnification in figure 22 which is the left half of figure 16, though from another slide; and in higher magnifications in figures 23 and 24. In figure 22 the character of the inflammatory reaction present is fairly well shown and the blue areas are also seen (*A*). At *B*, *C*, *D* are three small cancer buds; *D* is shown again in figure 23, *C* in figure 24 and *B* in figure 25. In figure 23, the preservation of the basal layer with bulging and lobulation is well shown. In figure 24 the changed direction of growth of the prickle cells, as far out as the granular layer, is very evident. As the bud enlarges, the basal layer remains intact and tends to arrange itself in the form of gland acini, as is already suggested in figures 23 and 24, and more clearly indicated in figure 25. This feature is a very puzzling one. I have never seen such structures in a fully developed carcinoma of the skin. Figure 26 seems to indicate that this gland-like arrangement soon disappears, the cavity filling with cells, sometimes very atypical, which mingle with the outer gland-like cells derived from the basal layer. In these proliferating prickle cells the early stages of an abundant pearl formation may be met with. The case corresponds, therefore, fairly well to the criteria laid down for cornifying carcinoma. Partly for this reason and partly because of the general character of the cells, I have become convinced that it belongs in that class.

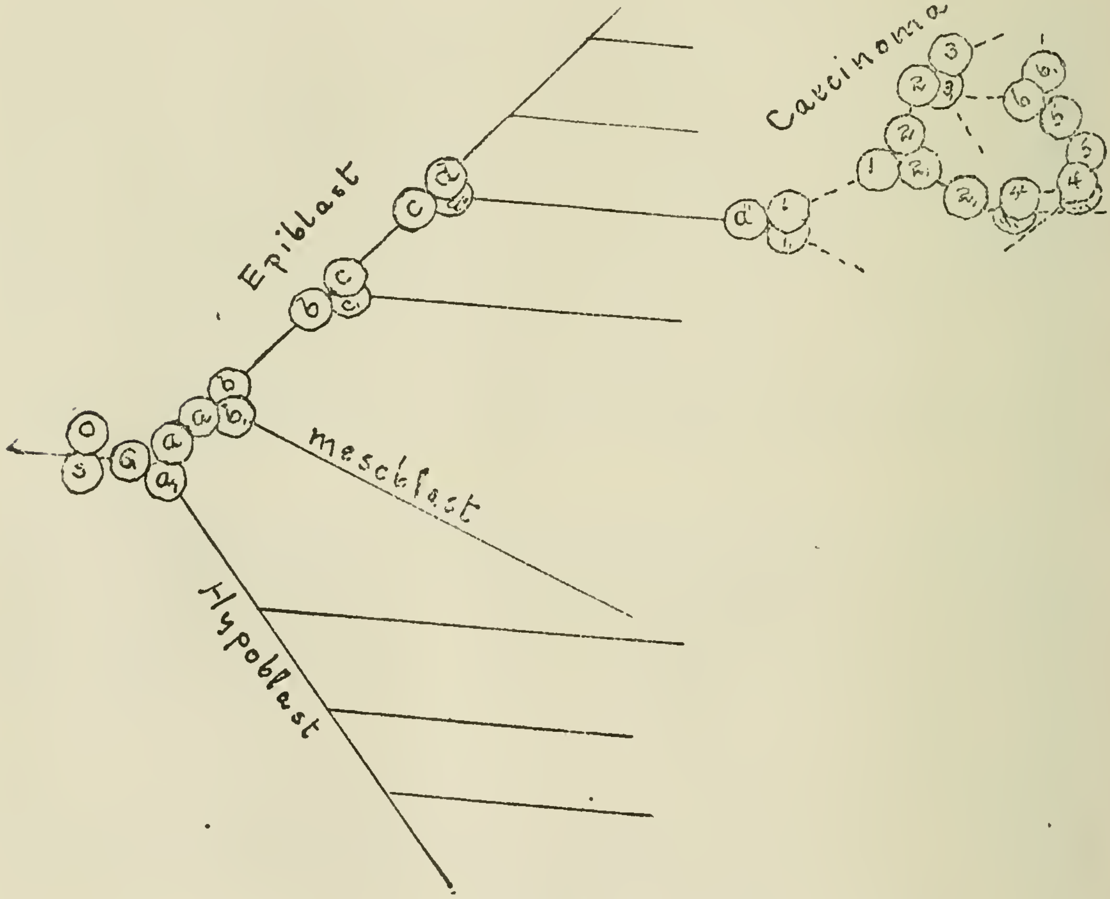
Returning now for a moment to figure 16 and the first mode of growth, it is apparent that the two modes are really one—a conclusion fully established by the fact that in some of the sections the difference between the two halves of figure 16 disappears and the epithelium everywhere presents the characters shown in the right half.

Finally, figure 27 shows a hair follicle clearly involved in the growth. That this is not due to secondary extension along the follicle is shown by the absence of any line of demarcation between the normal and cancerous cells.

The case is then one of cornifying carcinoma arising diffusely over a wide area of epidermis and also from hair follicles, and involving the prickle cells only. It is characterized especially by the growth of these cells within an intact basal layer, which at first enlarges to make room for the cancer cells, but later apparently disappears.

THEORETICAL CONSIDERATIONS

Whoever enters the field of speculation, knowingly ventures on very thin ice, and takes, as it were, his life in his hand. But I believe that in dealing with this subject, a record of observed facts can have but little value without an honest effort to understand and interpret those facts. It is not enough in other words merely to observe the apple falling. We must be not afraid to guess, since the accuracy of the guess measures the value of the whole effort.



TEXT FIG. 1

First, then, what is a cancer cell? This point I have recently discussed at some length and reached the conclusion that a cancer cell is a mutated somatic cell. It is not an embryonic cell, a cell presenting some sort of permanent, local infantilism. On the contrary, the cancer cell is a new cell, differing in its biologic properties from any cell at any time normally present in the body. Perhaps the point can more clearly be apprehended by reference to the accompanying diagram (text figure 1). Let the circles at the left represent the sexual cells which unite to form the germ cell. The germ cell multiplies and separates into two layers, epiblast and hypoblast (*a* and *a'*). In man the mesoblast develops from the former, as indicated (*b*). From the three layers thus formed the various organs and tissues develop.

Each new stage in development, indicated by a line branching off from the main trunk, is the result of an asymmetrical division; i.e., a cell, represented by a circle in the diagram, divides mitotically so as to produce daughter cells having unlike biologic properties, from *b*, *c* and *c'*; from *c*, *d* and *d'*, etc. One of these gives rise to the new tissue, the other continues in the same general direction of ontogenesis as the mother cell, though it also differs from the mother cell, to the same extent but in the opposite direction, from its twin. If we assume, as I think we must, that every property of the cell depends upon the presence within it of definite structures (genes or factors), it is difficult to conceive of any other way by which one sort of cell might arise from a different sort. When development is complete, this process of differentiation comes to an end, except as it is renewed from time to time, within narrowly circumscribed limits, in the processes of repair.

If the above is true it follows so obviously, I think, as to be axiomatic that a tumor cannot by any possibility be produced through the reversion of a mature cell to an embryonic stage of development. For no cell can go backward on its normal path of ontogenesis farther than the point at which it left the parent stem, since it cannot regain, out of nothing, the properties it lost in the unequal mitosis which gave it its origin. That is, *d'* cannot become *c*, since it cannot regain the properties originally lost to *d*. But at such a point of reversion the cell is nothing more than the normal embryonic cell of its kind—a fibroblast, for example, or a young, growing, but otherwise normal, epithelial cell. Certainly it is not a tumor cell.[1]

[1] It is not to be assumed that the separation of functions by unequal mitosis is absolutely complete; von Hansemann assumed that the new cell contains chief plasmae (or genes) (*Hauptplasmen*), for which I now prefer the term kinetic

If then, a tumor (a carcinoma, to take an extreme case) cannot arise by following its normal path of development in the reverse direction, it follows, since the carcinoma arises from an epithelial cell, that the departure from type must be in some other direction. That direction may be any you please, except the one. This conception of the process is illustrated in the diagram by the broken lines starting from the line which represents the epidermis. Anaplasia is this departure from type in a new direction (see my previous article).[2] "Anaplasia begins where normal ontogenesis leaves off" (von Hansemann). Moreover, since the direction of deviation from type is not constant (see above, cases 3 and 4), and since in the growing carcinoma ever new processes of deviation (i.e., new mutations) are or at least may be taking place, it follows that when established, the tumor as a whole is not necessarily a single entity; but it may be a jumble of entities, as I have sought to represent in the diagram, in which each broken line stands for a special variety of cell.

Thus far my argument follows von Hansemann's theory of anaplasia. But my conception of the process differs from his at this point, and I shall, therefore, develop here in greater detail a point of view which was touched lightly in the article previously cited. Von Hansemann believed that the "anaplastic" (tumor) cells arise by a process of unequal mitosis, analogous to that by which normal development takes place but differing from the latter in that the inequality of division involves whole

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genes; these are the actively functioning factors, and determine the normally expressed characters of the cells; and secondary genes (*Nebenplasmen*), which I now call latent genes. Under normal conditions these exercise no obvious influence on the life and form of the cell, but may do so under abnormal conditions. For example (I am not quoting von Hansemann now), they may determine the limits within which metaplasia can take place, and measure the capacity for reparative growth through reversion to the embryonic condition.

[2] In general I have avoided the use of the terms, "anaplasia" and "undifferentiation," in favor of the term, "mutation," not because the first two (particularly the first) are less serviceable than the last; but because the two former imply to most minds a process of reversion to an earlier, i. e., embryonic, state of existence; while the word, "mutation" leaves us free to imagine the cancer cell as one embarked on an entirely new career of *re*differentiation, the direction of which is anything you please, *except backward.*

chromosomes or perhaps groups of chromosomes. Of two such unequal cells the one which suffers a loss of chromosomes is "hypochromatic" and ultimately disappears. The other is "hyperchromatic" and constitutes the cancer cell.

Now while I by no means wish to deny the occurrence of asymmetry in von Hansemann's sense, I do not find it particularly helpful. For it is obvious that the hyperchromatic member of a pair has not, by virtue of such asymmetry alone, lost any factors or genes; on the contrary, it has gained those chromosomes and their contained genes, lost by the smaller cell. If we might assume that such an addition of chromosomes would cause a change in properties geometrically proportional to the extent of the addition, the case would be simple enough, for we could then suppose that the added genes had exalted certain characters by multiplying the number of identical genes present in the cell. But such an assumption is not justified, as I have shown in my earlier article. We may imagine if we choose that the addition of one or more chromosomes renders mutation easier and more likely to occur, by upsetting the smooth working of the mitotic process and by making what must be a really huge addition to the factors controlling the function and life of the cell. Their joint effect might find myriad expressions.

It is evident that I have enjoyed one decided advantage over readers of this essay, in that I have been able to select for illustration those features which seemed to me to favor most my own views. Doubtless also in spite of the best will in the world to the contrary, the selection of fields for discussion and illustration has been influenced by my personal attitude. On the other hand it is presumably not of prime importance whether my own explanations for the varied phenomena of growth observed are accurate to the last detail. I feel safe in saying that no one can possibly differ with me more earnestly or in more directions than I have differed with myself from time to time in the course of these studies. But it is important that in a series of four cases of carcinoma, of the same general type, no two are exactly alike in the details of origin and growth. There is no apparent reason for assuming that these differences are the result of adaptation to differences of environment. On the contrary I believe that the whole theory of adaptation has been sadly overworked and that it is at least as rational to assume, for example, that the mole and the blind fish live in darkness because they are blind, as it is to assume that they are blind because they live in darkness. My own mind rests, more contentedly than on either of these notions, on the assumption that they live in darkness because they have lost the factor for heliotropism. My article, already cited, discusses this question at greater length.

I prefer, therefore, to regard these differences in behavior as proof of corresponding differences in the inner structure and life of the cell. The cells of a tumor, like the cells of the body, are subject to laws of growth imposed more from within than without. If we apply the law that "cells feed, but are not fed" (which like many laws is valid only within certain limits) we can see that a quantitative or qualitative deficiency or excess of food, for example, while it might cause various diseases of the cancer cell, could not in and of itself, affect the fundamental biologic laws controlling the cell, beyond contributing, so to speak, to the delinquency of the cell by serving as the occasion, but not the cause, of new mutations. Similarly with regard to pressure effects. Pressure might, to be sure, modify the external shape of the cell as a tight bandage modifies the shape of a foot. But the foot remains a foot, normal so far as the intrinsic laws of its existence are concerned. Moreover, such an effect is usually easy to recognize through its spacial relation to its cause, and the cancer cell is able to escape readily from such effects, by destroying the cells that press upon it. Granting the validity of these considerations we can, I think, see in the changing behavior of the cells definite ocular proof of changing constitution.

It seems to me, therefore, that mutation of the cell (whatever that may be), is the essential element in the origin of the cancer cell. Nor need we imagine that the change from a normal to a malignant cell necessarily takes place at a single step, as Minerva sprang full panopled from the head of Jove. There are plenty of facts to indicate, as von Hansemann among others long ago pointed out, that the biologic state of a growing cancer is anything but stationary and that functions present in the parent tissue may become progressively changed or lost.

It is, of course, exactly this fact that renders the theory of mutation applicable to all tumors, whether benign or malignant, whether they arise from a cell rest or from an anlage normally intercalated in the tissues. An adenoma or a fibroma remains an adenoma or a fibroma until a further mutation or series of mutations converts it into a carcinoma or a sarcoma, respectively. An osteochondrofibroma, for example, may arise from a homogeneous tissue whenever that tissue retains as "latent genes" (suppressed by inhibiting factors in its mature state) the factors necessary to the production of the several sorts of tissue present in the tumor; the loss of one or other of the inhibiting factors in this or that area determining the growth of such areas in the corresponding direction. We are also afforded a clear insight into the otherwise puzzling fact that a tumor belonging to one embryonic layer can never become a tumor belonging to a different layer. If we regard the tumor cell as the result of undifferentiation backward along the path it followed in normal development, we must assume its ability to follow that path beyond the point where it separated from the cell that gave it origin. If it can do this, there is no great reason why it might not go still farther along the same path to and beyond the point where the embryonic layers formed, and thus become, from, for example, an epithelial cell, an earlier indifferent cell manifesting characters belonging to other layers. This we know never happens, and this becomes readily comprehensible when we regard the tumor cell as the product of re-differentiation along new paths, with characters dependent on the total effect of the factors present, which, however modified by mutation, cannot produce the characters of other embryonic layers, since none of these were present in the parent cell. Tumors containing elements of more than one layer can arise only from pluri- or totipotential cells, or from a cell rest isolated later, and containing cells belonging to each of the layers present in the tumor.

Indeed, the theory, whose acceptance no doubt demands an act of faith, is as flexible to serve varied purposes as is the side-chain theory in its particular domain.

The cancer cell, then, or, more broadly, the tumor cell may be defined as a cell arising by mutation from a normal (though perhaps displaced as a cell rest) somatic cell. So long as the new species of cell thus produced continues to grow without undergoing further mutation, the character of the tumor will remain constant; but when new mutations occur, from time to time and here and there, new sorts of tissue arise to correspond.

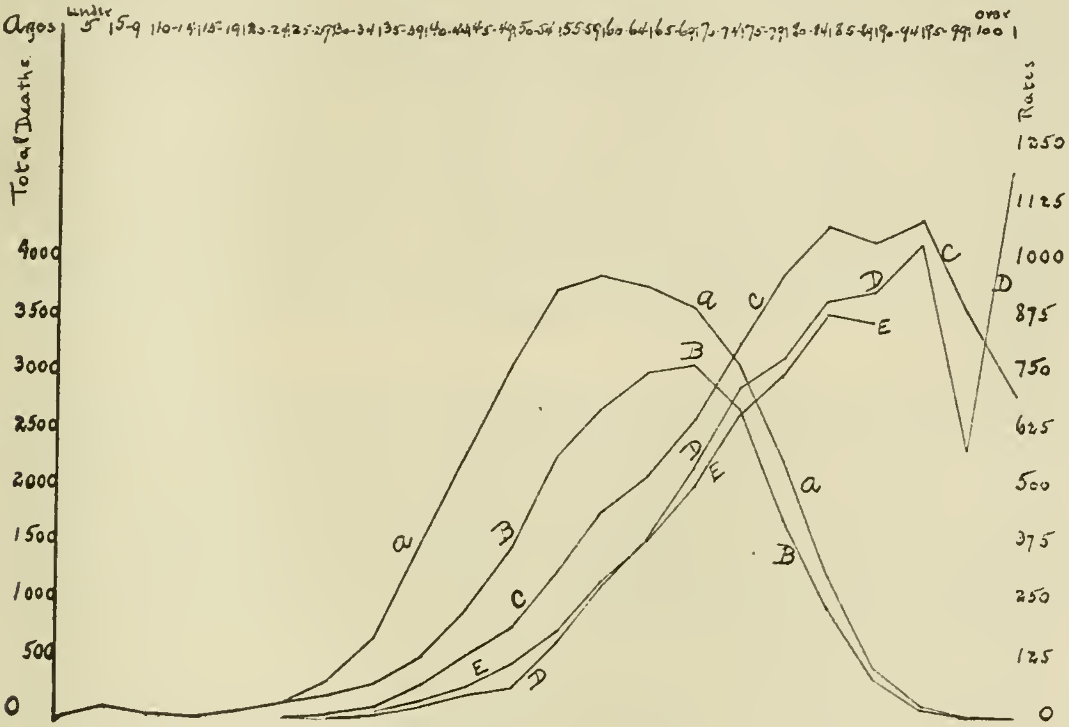
This leads me to protest against the familiar habit of describing or even defining this or that tumor as "tending to differentiate as" or as "seeking to produce" this or that parent tissue. If we accept the mutation theory, we must believe that a tumor is not a mere abortive effort to produce something else than itself. It is what it is by virtue of the properties inherent in its factors and can be properly regarded as growing only in obedience to its own laws of growth and "tending to differentiate as" just one thing, namely, itself. The familiar phraseology is really as absurd as it would be to speak of a pig, seeing its two-toed hoof, as tending to differentiate as a cow, of which ideal it falls short by its inability to grow horns and regurgitate its food.

Second, when then shall we say that a cancer has begun? It is apparent that a precise answer cannot be given, but I believe we can get much nearer a correct answer than that represented by the familiar view, that carcinoma exists only when the process of infiltrative growth has made it clearly recognizable under the microscope. Analogies are dangerous, but sometimes useful. When shall we say that tuberculosis has begun? When it is diagnosed? Clearly such a view is nonsense. Then, when if looked for, definite histologic evidence of it could be obtained? Clearly again there must in every case be a stage when changes exist so delicate that even the most skilled observer could not recognize them. It is obvious that the disease must be regarded as having begun at the moment when the bacillus, having gained entrance into the body, has set in motion that process, interruptable at any step to be sure, by which in the ordinary, "normal" course of events, recognizable degrees and forms of the disease will be produced. This reasoning may quite logically be applied to the case of carcinoma. It is apparent that the capacity for and tendency to invasive growth must be acquired before such growth can take place; and since invasive growth is one of the characters that define the cancer cell, it is clear that the cancerous process is present before invasive growth begins, and from the moment when the series of changes leading to development of cancer is set in motion, beginning perhaps a long time before recognizable carcinoma tissue is present. Whether we can recognize a carcinoma or ever hope to recognize it at such a stage, has in the language of the old song "nothing to do with the case." That the ability to grow invasively does not of itself constitute carcinoma is clearly shown by such phenomena as the pseudocarcinoma or carcinoid condition of the appendix.

Third, the etiology of cancer becomes then the etiology of mutation. The law that appositional growth never takes place (Ribbert's *Aussichherauswachsen*), precludes the idea of a specific infectious agent, in the ordinary sense, for we must either assume that such an agent dies after having started the cancerous process but before invasion begins (a notion which, by the way, admits my contention that the cancerous disease is present before the cancer is there) or else that there exists a multitude of specific agents, one for each kind of tumor and for each kind of tissue; for example, one agent for carcinoma of the stomach and another for carcinoma of the liver. On the other hand, the development of cancer following the action of a considerable variety of nonspecific irritations (lupus, gastric ulcer, arsenic, paraffin, soot, tar, wind and weather, as in seaman's cancer) some of which (*x*-ray, radium) are known to have a specific action on the mitotic process, furnishes us with a much simpler and more comprehensible viewpoint. Whenever such an irritant excites over a long period, constantly renewed and constantly defeated efforts at repair, an enormously increased opportunity is obviously afforded for such an accident to occur during the mitotic process, as would produce the particular mutation required.

Ribbert, as is well known, has ascribed great importance to the degenerative and inflammatory changes usually present in the neighboring connective tissue. My cases show that these changes are not always present. Inflammatory infiltration of a certain degree may antedate *visible* changes in the epithelium, but its intensity and extent increase up to a certain point, pari passu, with the further growth and spread of the tumor. The commonly observed absence of an inflammatory reaction in carcinoma of the fundus of the uterus can be adequately explained perhaps on the assumption that the location and character of the growth is such as to afford free drainage for the product of its own abnormal metabolism, so that no absorption of them takes place. That portion of the change which is not the result, rather than the cause, of the cancer may be quite reasonably explained as a pure coincidence, since it apparently extends over a vastly greater area than that involved in the tumor growth, and may be present where no tumor exists (cf. Unna, loc. cit.). Or we may regard these changes as part and parcel of the cancer process, as the result not the cause, of changes occurring in the epithelium, out of which the cancer eventually arises. At the most such changes might afford an increased opportunity for the sort of accident just mentioned, in so far as they antedate the cancerous process and in so far as their presence implies the presence of anomalies of metabolism.

I have already urged (loc. cit.) the possible importance of hybridization and mongrelization in the same direction. The same general considerations apply to the effect of old age. Attention has recently been drawn to the fact that there is no such thing as a "cancer age." For although it is true that the total number of deaths from cancer is greatest in the decades on either side of the (male as well as female) menopause, the cancer rate increases steadily throughout life and is greatest in those one hundred years old or over (text-fig. 2). In this figure the line *A* represents the total number of deaths of women from cancer by ages, in the United States registration area in 1914, as given in the Census Bureau's mortality tables for that year. Line *B* gives the deaths of men. Before age thirty and after age one hundred, the number of deaths for each sex, while not identical, is so nearly equal that the difference cannot be indicated in a graph of the scale I have used and no separation in these age limits is attempted. Line *C* is the death rate per 100,000 for women and line *D* the rate for men. Here the rate before age twenty-five for women and age thirty for men is so low that it cannot be indicated with any accuracy, and is therefore omitted. The curves show a slight absolute drop in the rate for women in the age group from eighty-five to eighty-nine, and a relative drop for men in the same age group. There is also a decided fall in the death rate for women after age ninety-five, and a sharp drop in the rate for men between ninety-five and ninety-nine, with a subsequent sharp rise in the group over one hundred to the highest point in the curve. These curves were obtained by dividing the total number of deaths as given for 1914, by the total number living in each group, in the same area, as given for 1910. They are therefore "crude" rates, but could not be corrected since the Census Bureau was unable to furnish corrected populations for that year. I can explain the drops in the rates



Text Fig. 2

Curve A = total deaths in women, by ages, from cancer in 1914.
Curve B = total deaths in men, by ages, from cancer in 1914.
Curve C = death rate per 100,000 in women, from cancer in 1914.
Curve D = death rate per 100,000 in men, from cancer in 1914.
Curve E = death rate per 100,000, both sexes, from cancer in 1914. Figures furnished by Bureau of the Census.

above mentioned only on the assumption that they are due either to an unexpected change in the total number living in the age groups involved or, more probably, to the fact that the total number living in these age groups and the number of deaths were so small as to produce wholly misleading results. Rates determined in the same way for the year 1913 give a like result. Line *E* was obtained by reducing to graph form a table giving the joint rate for both sexes, published by the Census Bureau after I had prepared the rest of the figure. That table extends only from age thirty-five to age ninety. Between the ages of thirty-five and sixty-five it lies, as would be expected, between the lines for the sexes as I plotted them. After age sixty-five it lies below both my curves. This result is perhaps due to the fact that my rates are, as already mentioned, crude rates, while the Bureau's are doubtless corrected. The difference is wholly unimportant in the present discussion. It will be noted that the Bureau's table also shows a slight drop in the rate after age eighty-five.

The fact that the cancer rate rises throughout life is in harmony with the idea that the likelihood of a disturbance in the enormously delicate and complicated process of mitosis increases the longer we live, in accordance with the law of chance. Possibly the increasing sluggishness of function incidental to old age adds a favoring factor.

The same applies to the parakeratotic process to which also Ribbert attaches importance. It is at least as rational to assume that these are an expression of a mutative change in the life of the cells, representing a step in the process to which the cancer owes its origin.

SUMMARY

1. The carcinoma cell is a new variety (or better, species) of cell, arising by somatic mutation from a normal cell existing either as a cell rest, or normally intercalated in the tissues of the host, and retaining in almost every case enough of the properties of the parent cell to render its point of origin recognizable within certain limits.

2. The cause of such mutative changes can only be surmised; but apparently they are favored by hybridization and mongrelization, and are more immediately brought about by chronic irritations which maintain long continued efforts at repair—particularly when such irritants exercise, as in the case of *x*-ray and radium, a specific power to interfere with the mitotic process.

3. Such mutations are apt to recur from time to time in the growing tumor, bringing about a progressive loss of the original character of the mother cell, and change in the clinical behavior of the tumor.

4. It follows from 3, that in many cases the biologic properties of a carcinoma are not constant or uniform, but vary from time to time and region to region of the tumor.

5. It further follows from 3 and 4, that since the histogenesis of a carcinoma is a gradual process, the real beginning of this process must actually antedate visible changes in the cell, by perhaps a long period.

6. The particular characteristics of squamous-cell carcinomata do not depend upon the tissue of origin, but upon the character of the responsible mutative change.

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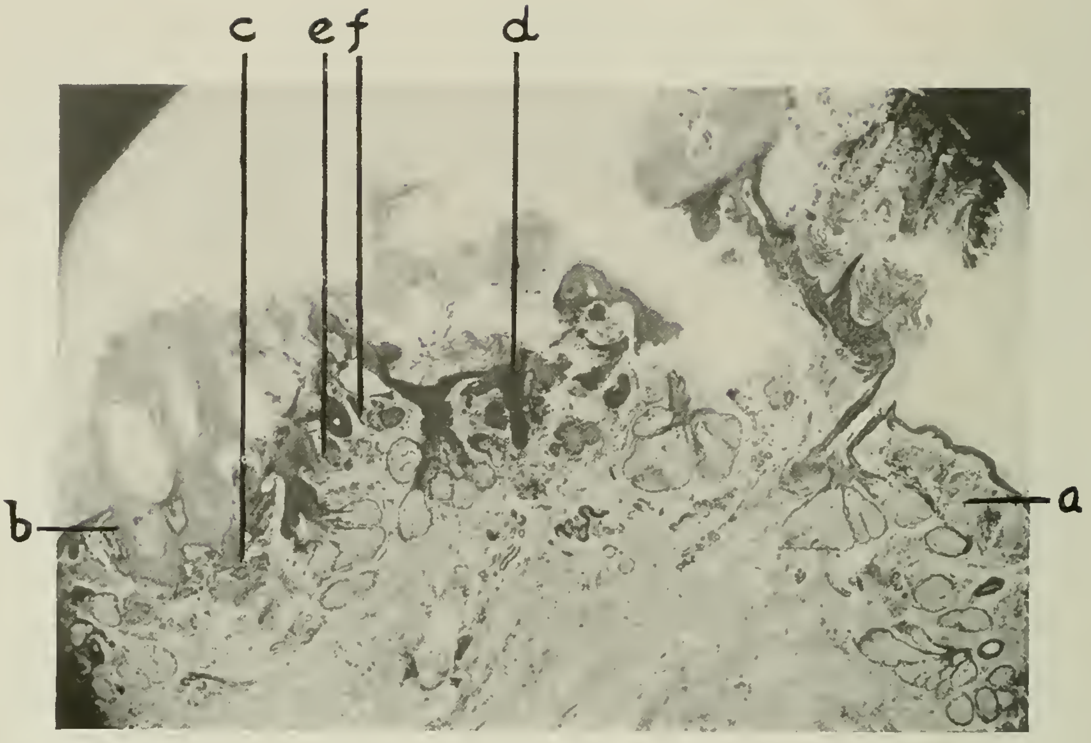
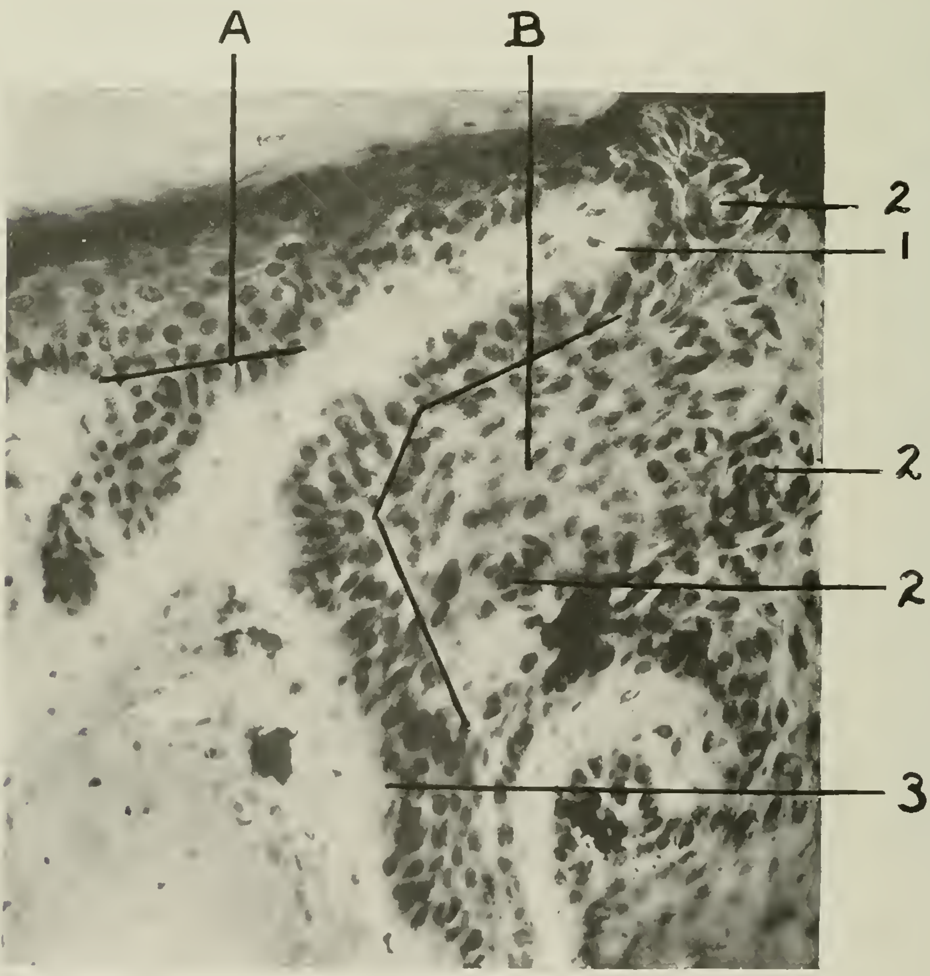


Fig. 1



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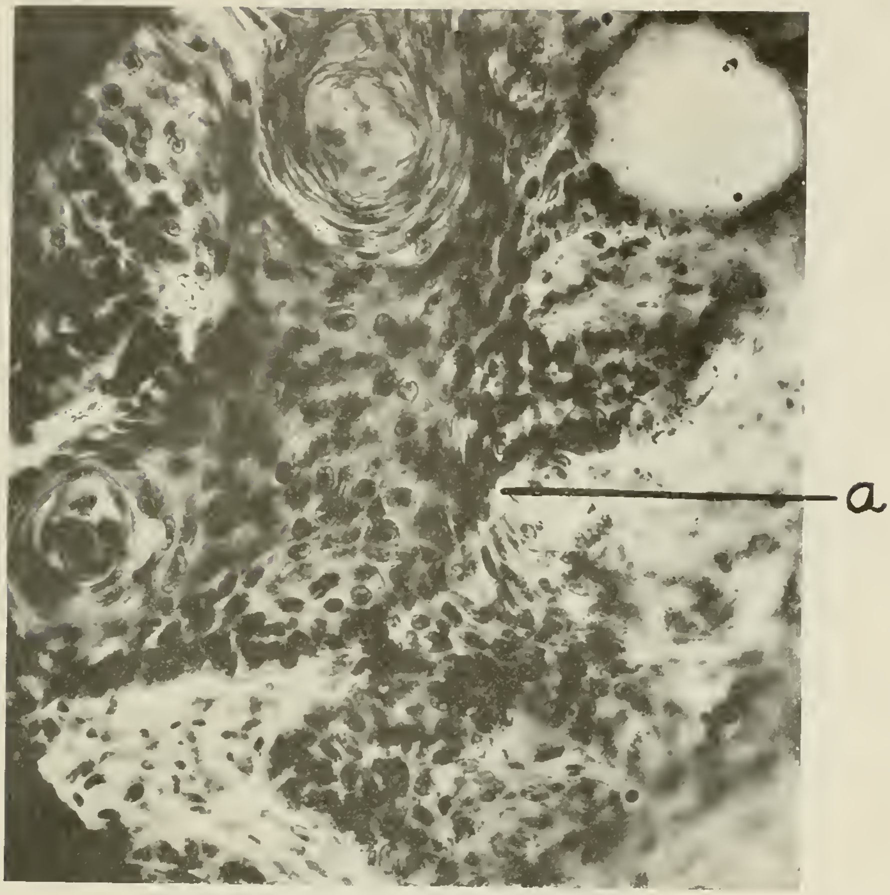

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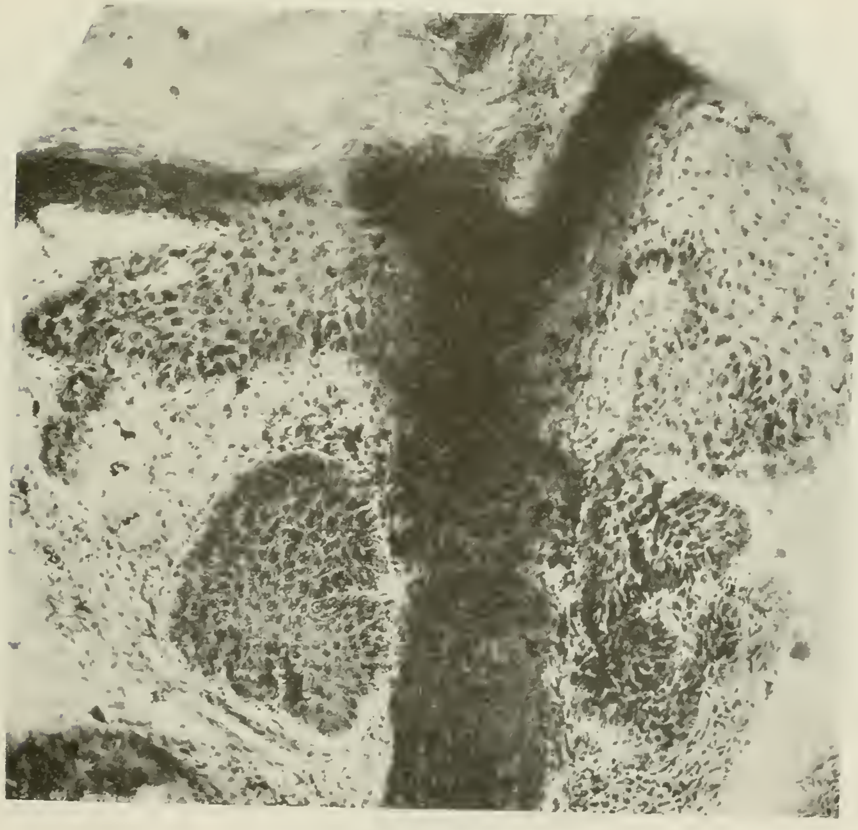

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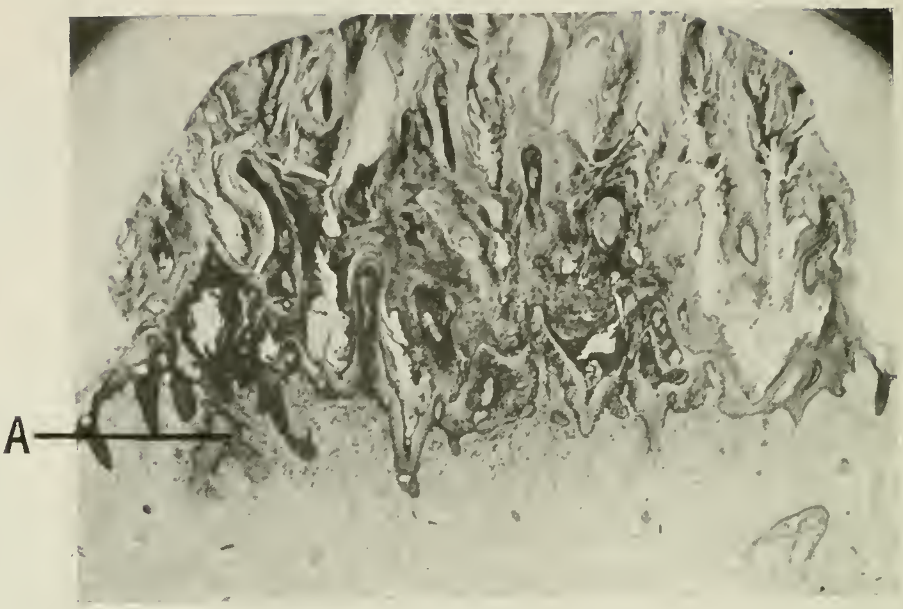

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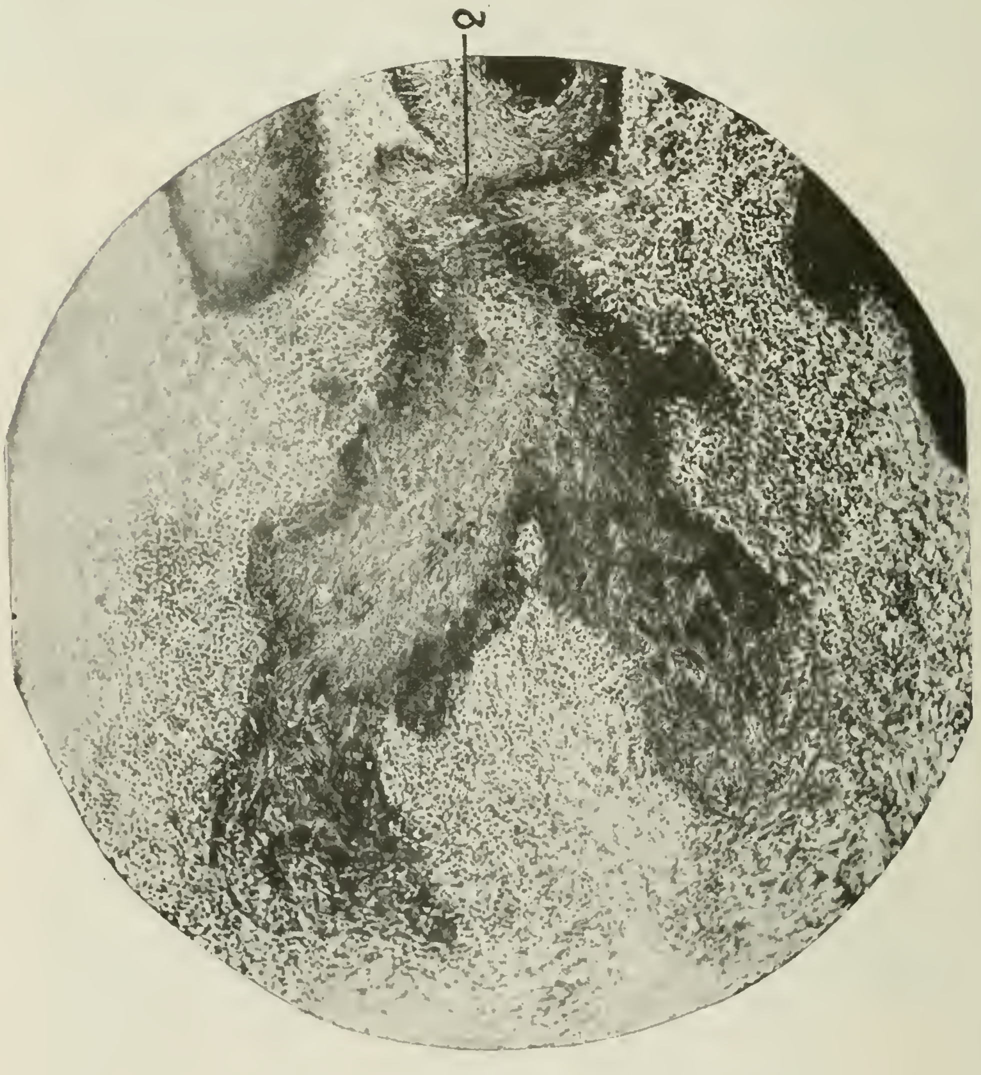

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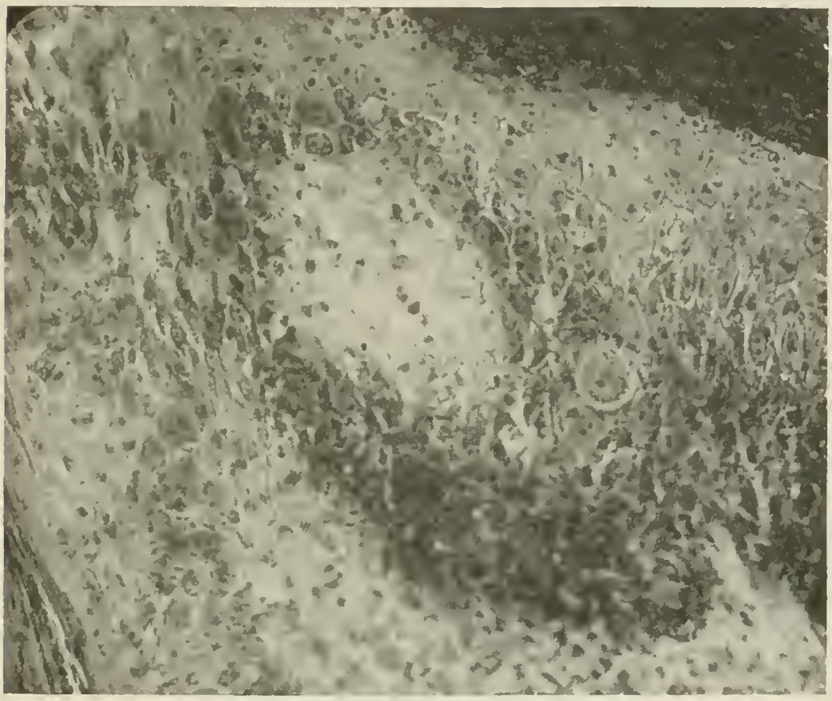

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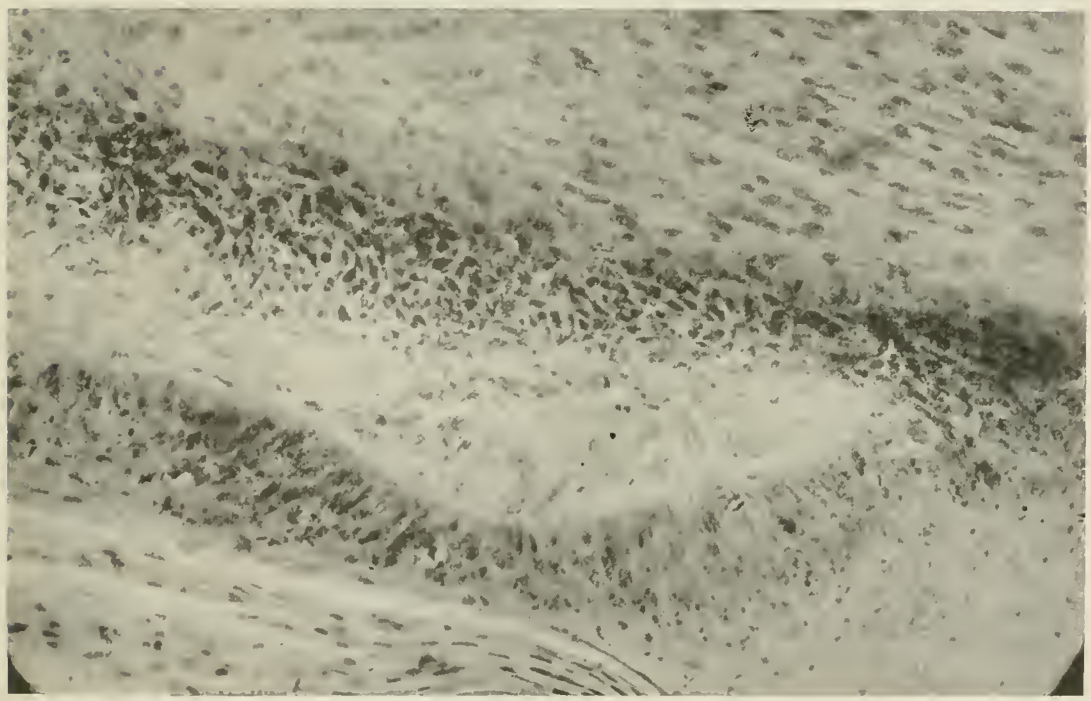

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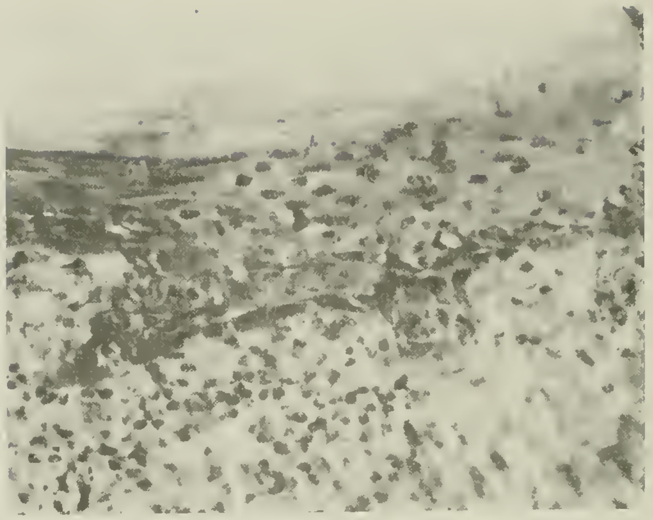

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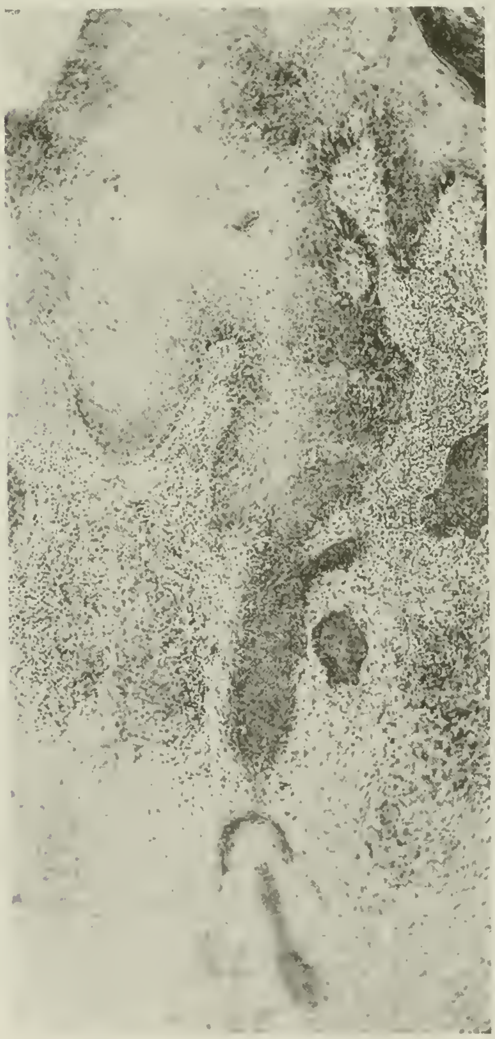
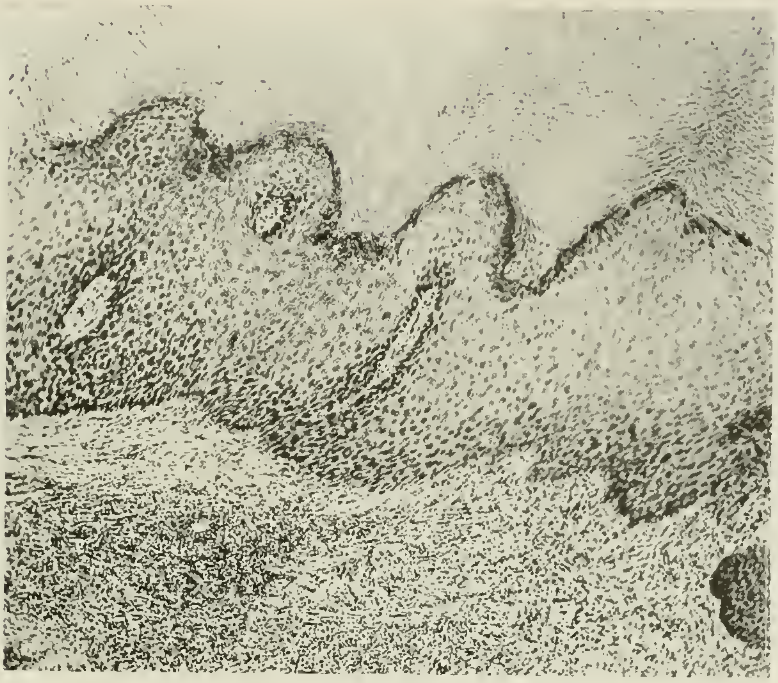


Fig. 10



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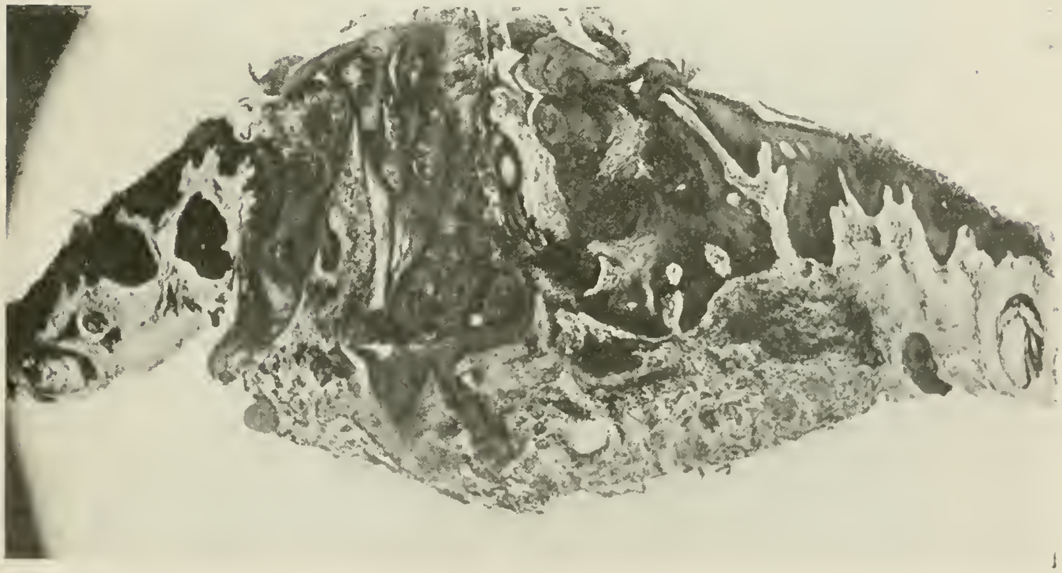

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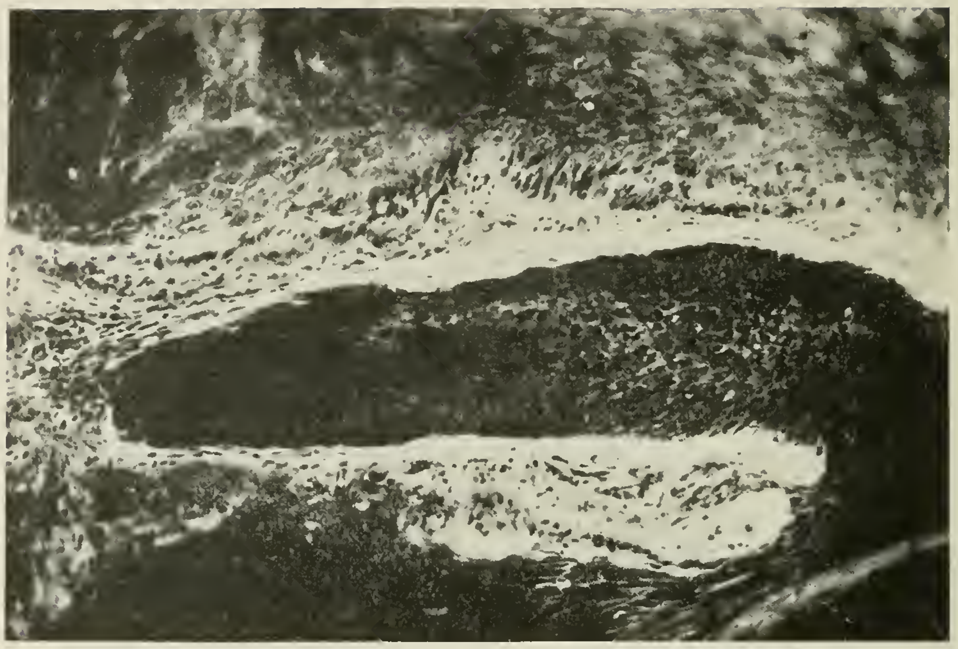

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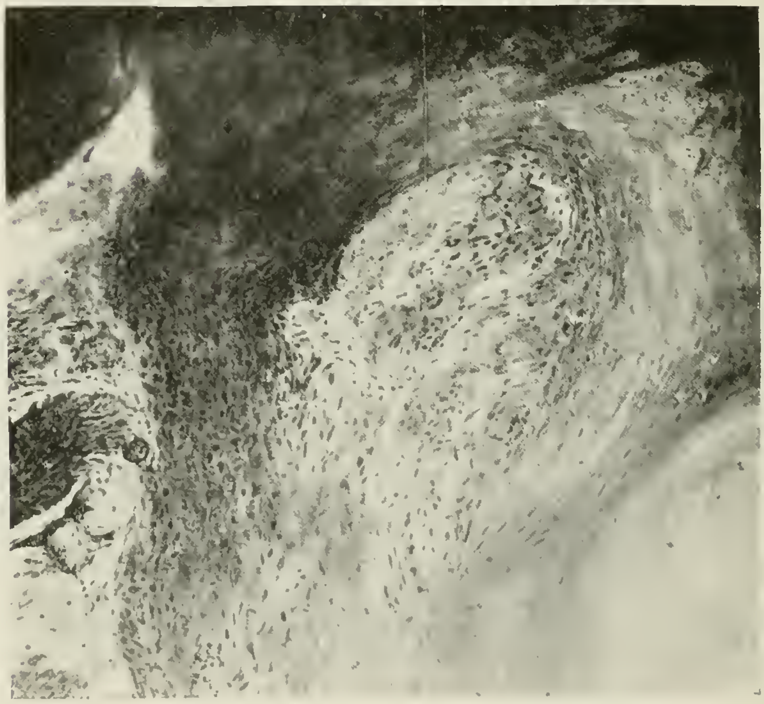
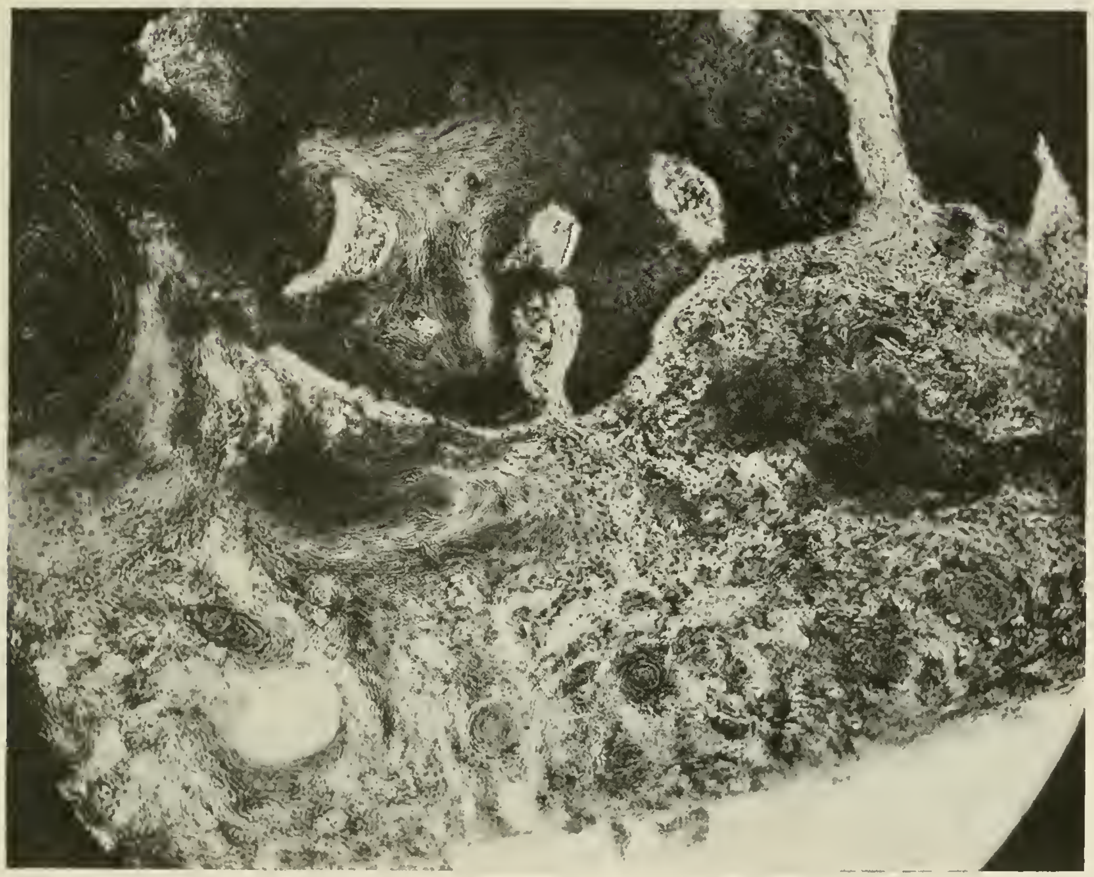


Fig. 14



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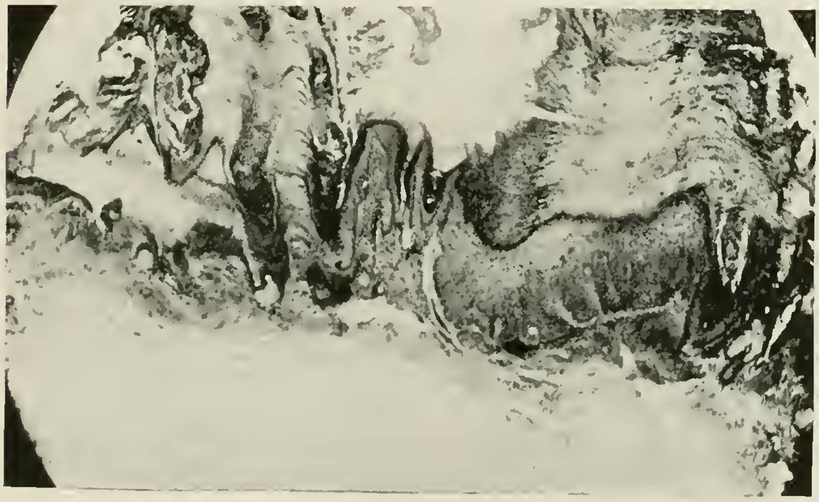

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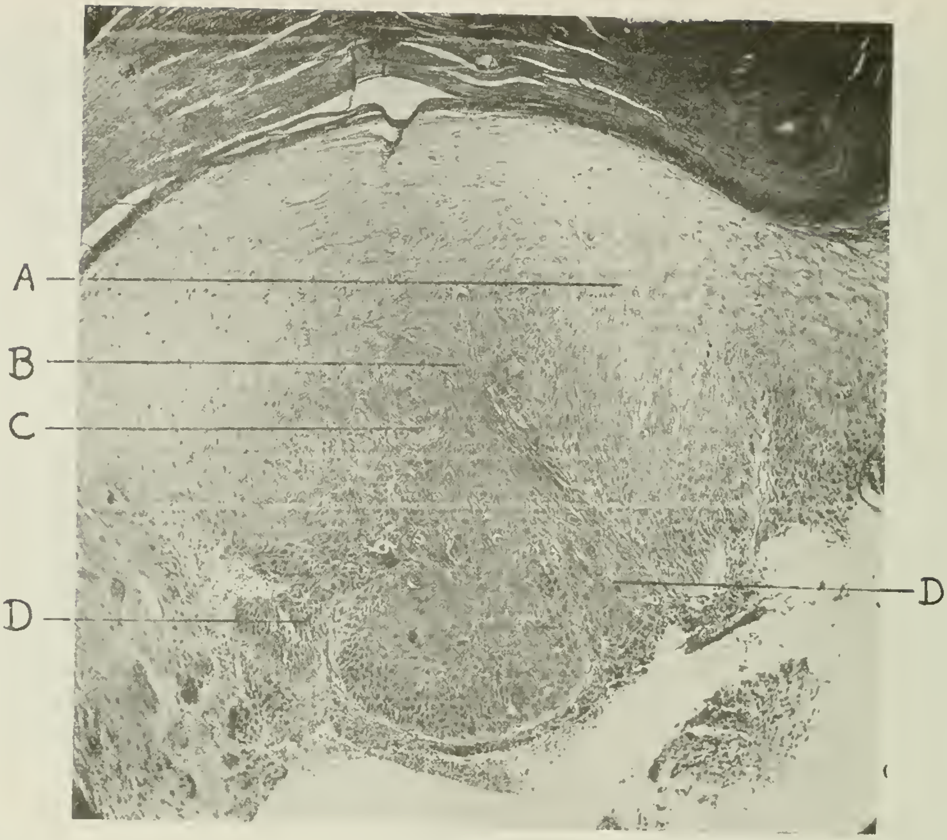

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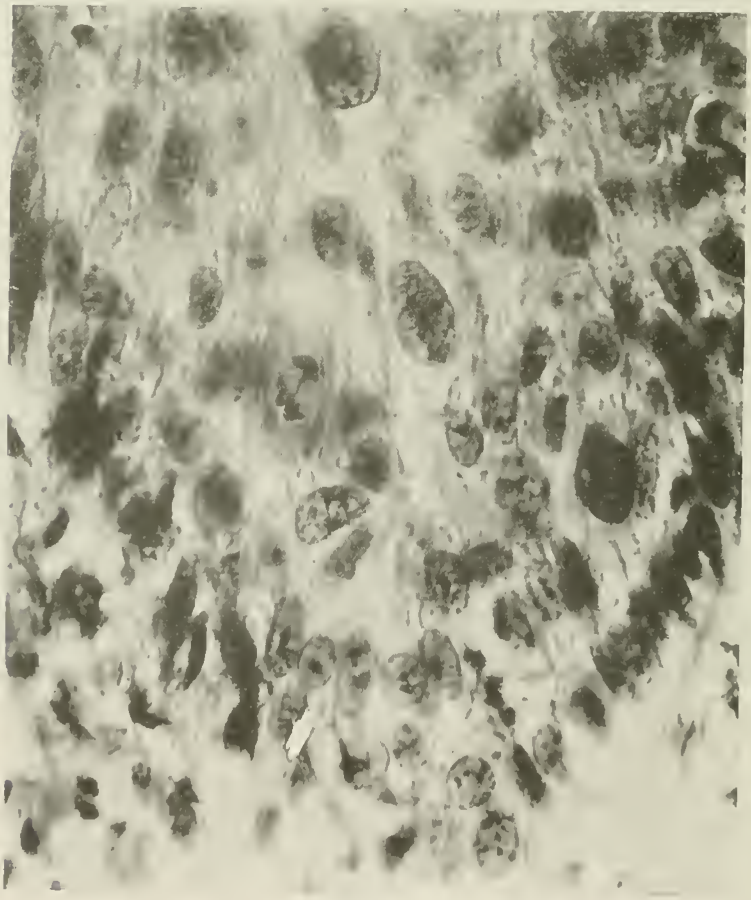

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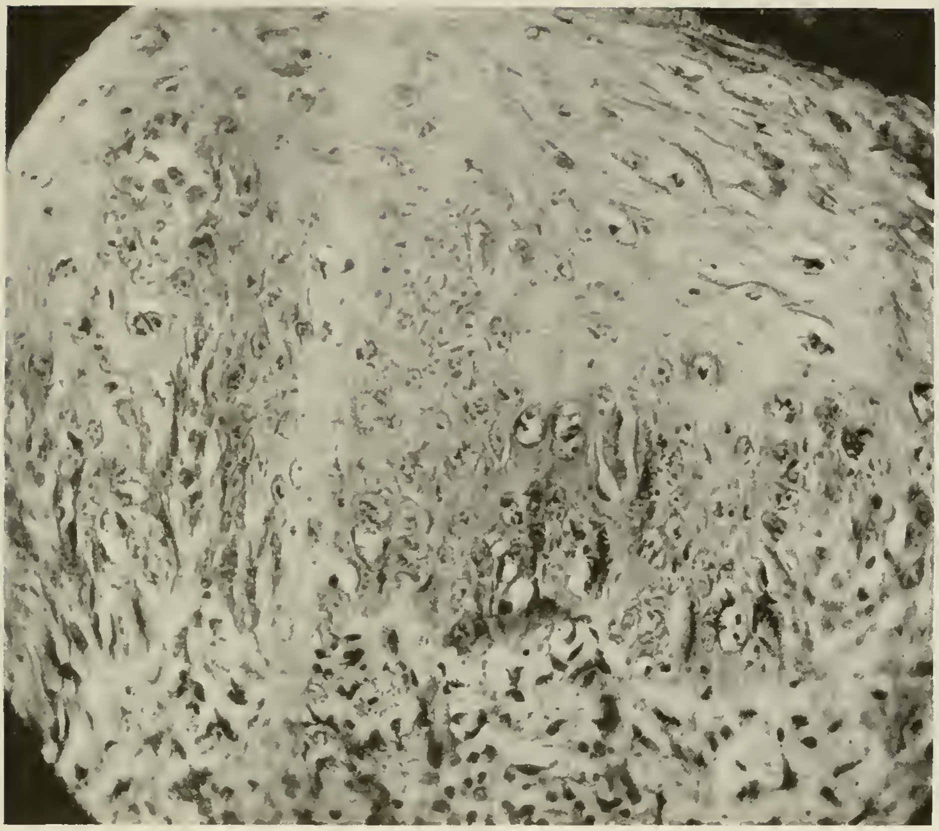

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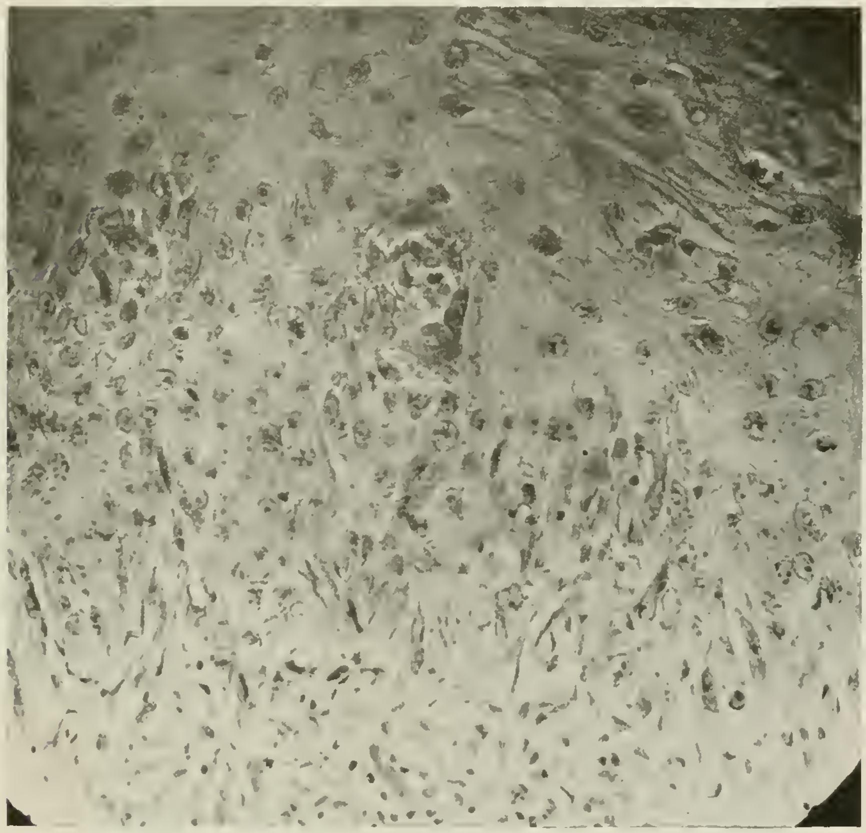

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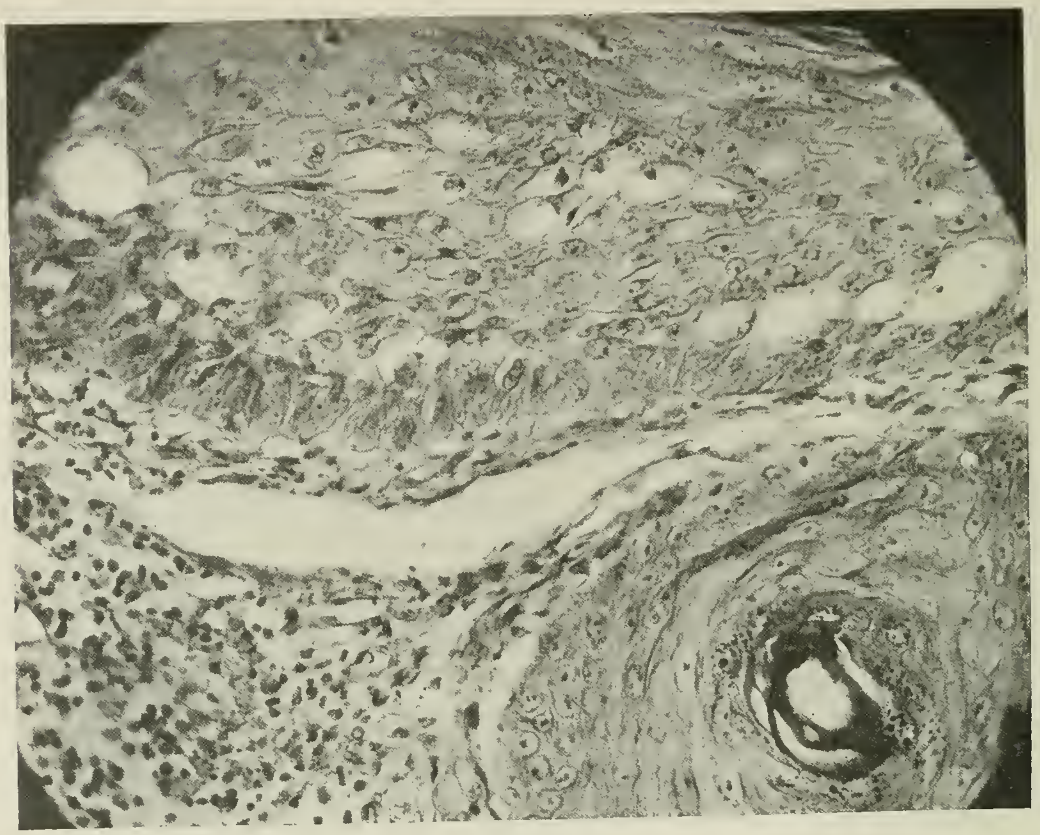

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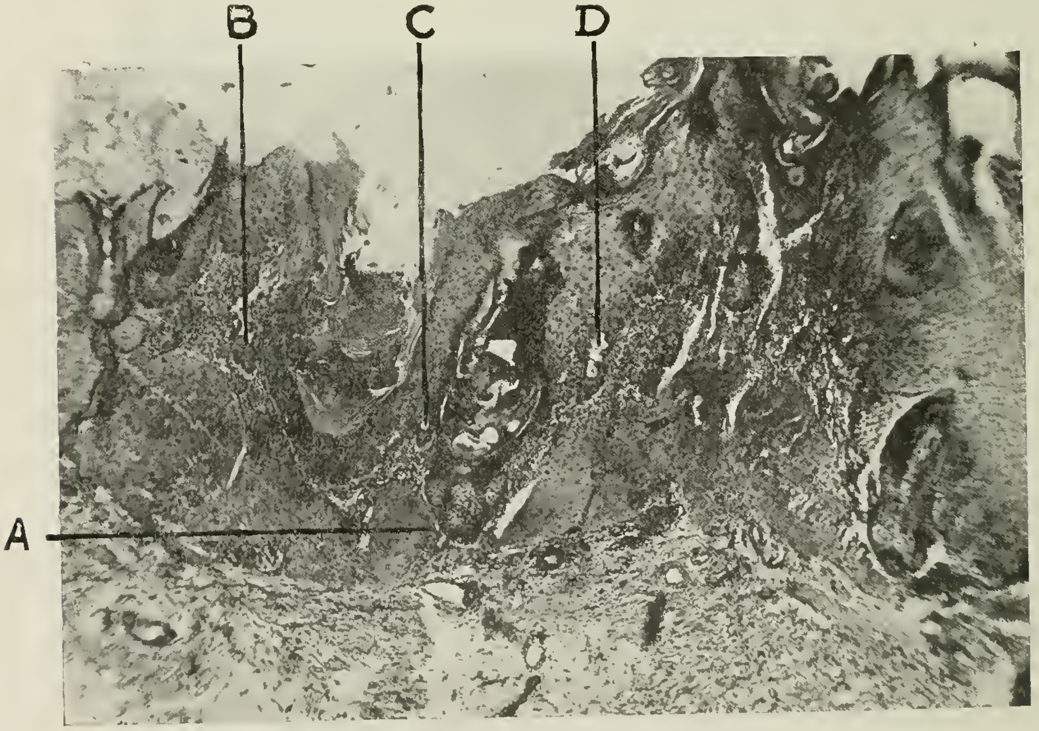

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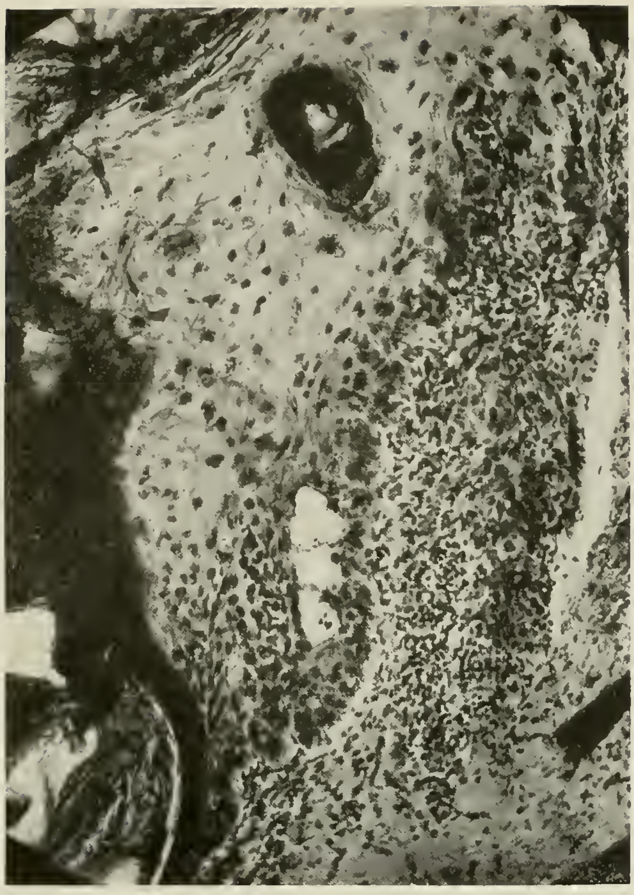

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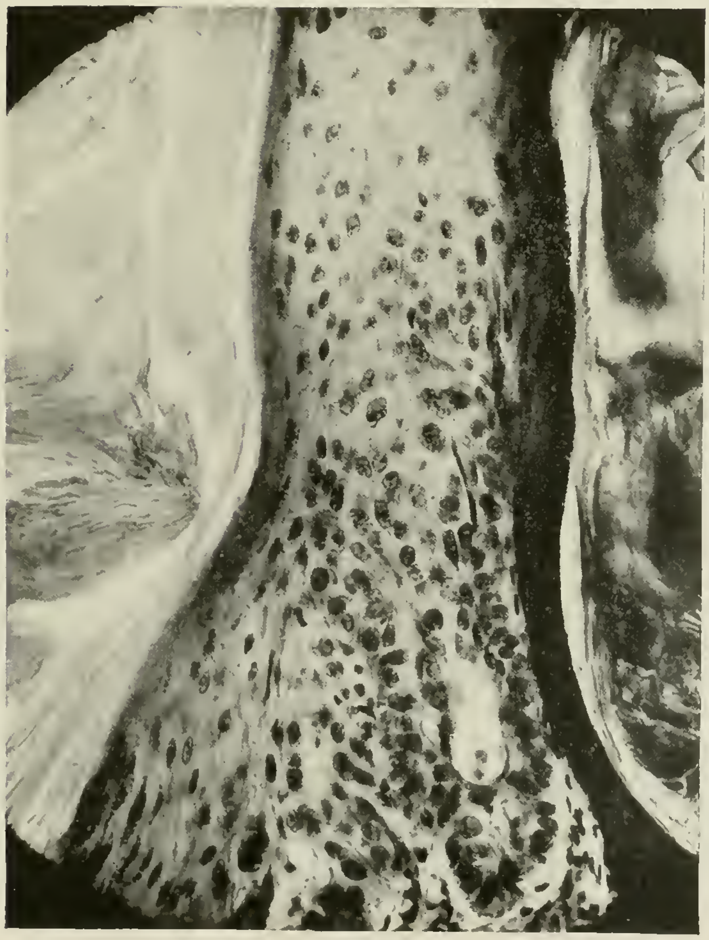

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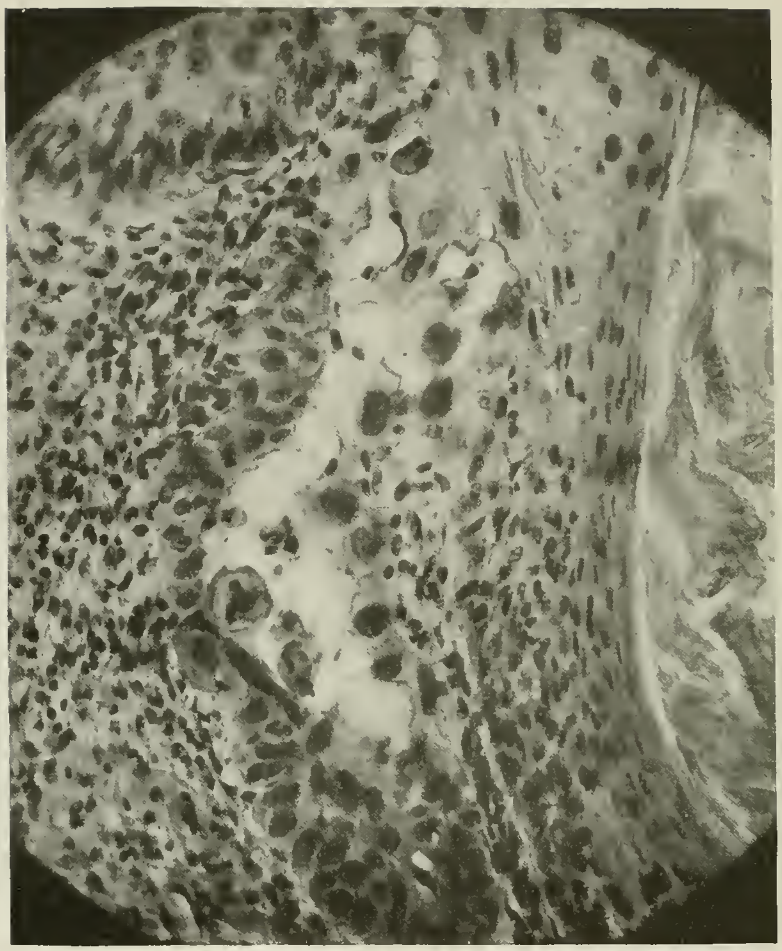

Fig. 25

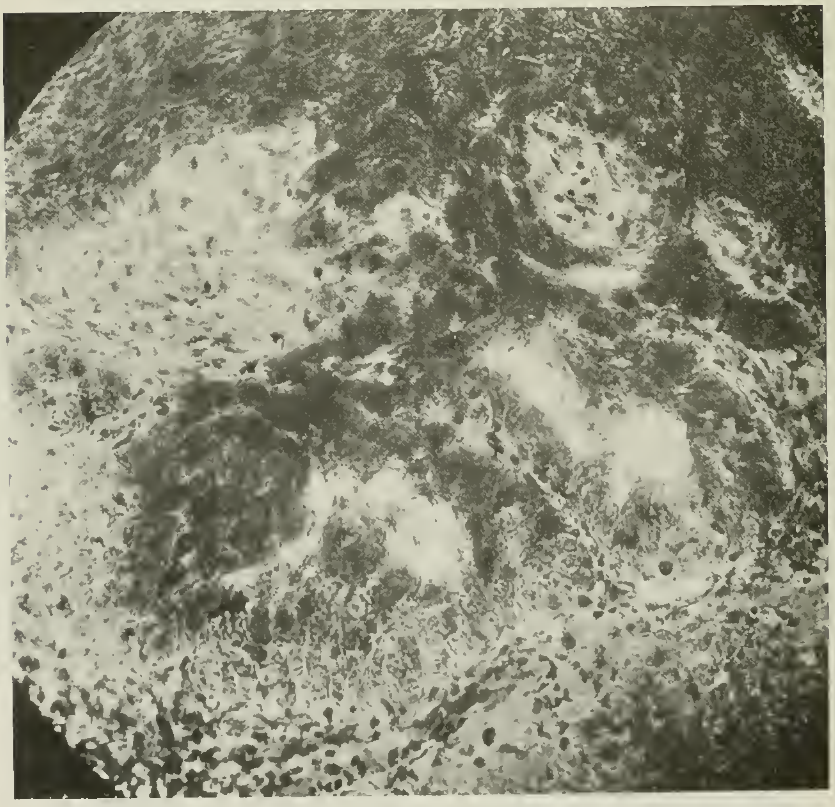

Fig. 26

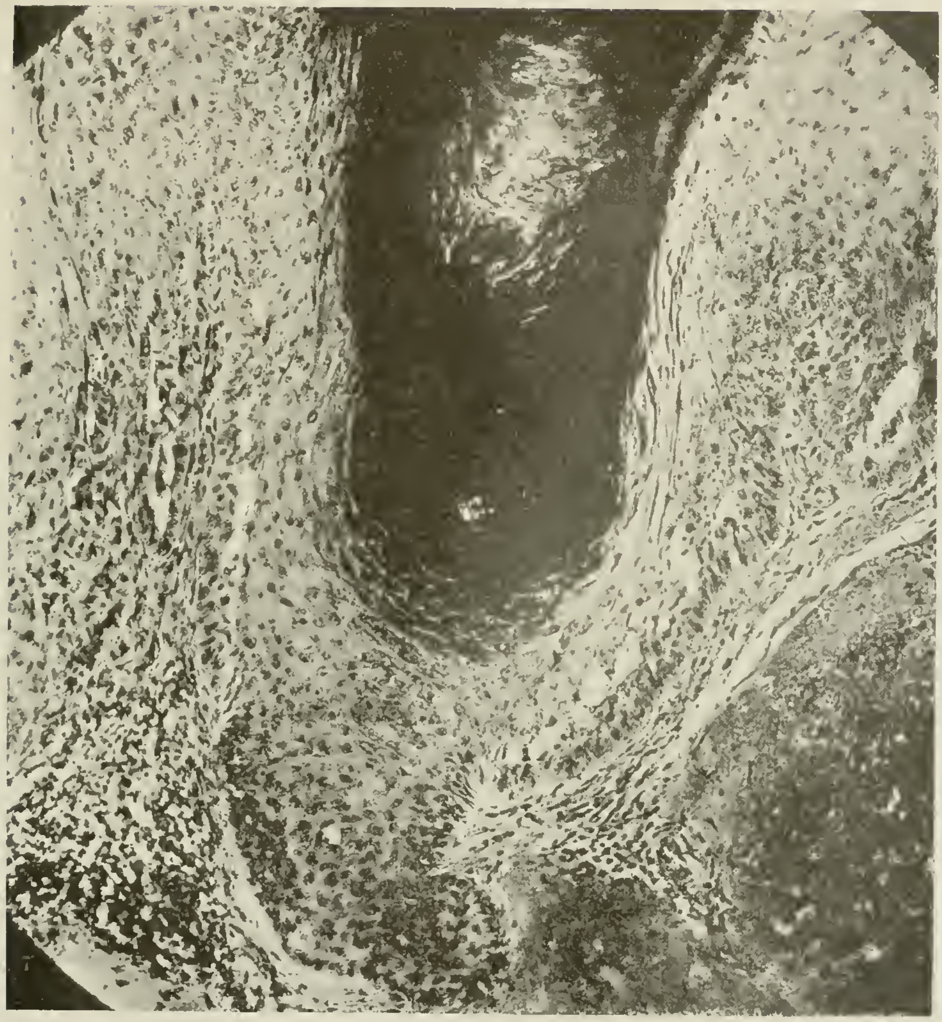

FIG. 27

ON THE CALCIUM CONTENT OF THE BLOOD WITH SPECIAL REFERENCE TO CANCER

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In his studies on the chemistry of malignant growths, Beebe (1) found that old degenerated carcinomata and sarcomata contained much more calcium than did young, rapidly growing tumors. Likewise, Clowes and Frisbie (2) observed a low calcium and high potassium content in rapidly growing mouse tumors, and the reverse in tumors of slow growth. These observations suggested the possibility of a disturbance of calcium metabolism in neoplastic disease; and, with a view to demonstrating such a disturbance, the determination of the blood calcium was undertaken in a series of cancer cases.

As a basis for comparison with the values obtained in cancer, the calcium content of the blood of a number of patients presenting no evidence of malignant disease, was ascertained.

The method employed was that of Halverson and Bergeim (3). The technique of these authors was closely followed, except that the protein precipitation was done in a 50 cc. centrifuge tube and centrifugalization resorted to in order to facilitate removal of the protein precipitate. Duplicates were done in all cases.

The data of thirty-four patients with malignant disease are presented in table 1. The calcium content of the blood plasma varied from 5.87 to 15.05 mgm. per 100 cc., the average being 9.41 mgm. If the type and location of the tumor are disregarded, the calcium concentration of the plasma of the male is slightly lower than that of the female, the mean figure for the former being 9.42 mgm., for the latter 10.21 mgm. These average values may be considered to be within normal limits, Howland and Marriott (4) having found from 10 to 11 mgm. per 100 cc., and

Halverson, Mohler, and Bergeim (5) from 9 to 11 mgm. in a series of normal individuals. In 64 per cent of the inoperable abdominal cases, the calcium content exceeded 10 mgm., but a truly characteristic calcium value for any definite type or location of

TABLE 1

	SEX	DIAGNOSIS	CALCIUM PER 100 CC.
			mgm.
1	M	Epithelioma of face	8.28
2	M	Epithelioma of face	5.87
3	M	Epithelioma of back	10.96
4	F	Carcinoma of breast	8.04
5	F	Carcinoma of breast	8.20
6	F	Carcinoma of breast	9.35
7	F	Carcinoma of breast	8.50
8	M	Abdominal carcinoma*	11.60
9	M	Abdominal carcinoma	10.41
10	M	Abdominal carcinoma	15.05
11	F	Abdominal carcinoma	14.24
12	M	Abdominal carcinoma	10.30
13	F	Abdominal carcinoma	11.67
14	M	Abdominal carcinoma	10.41
15	F	Abdominal carcinoma	13.40
16	F	Abdominal carcinoma	10.79
17	M	Abdominal carcinoma	10.71
18	M	Abdominal carcinoma	9.08
19	M	Abdominal carcinoma	9.11
20	M	Abdominal carcinoma	6.02
21	M	Abdominal carcinoma	6.18
22	M	Abdominal carcinoma	7.10
23	M	Abdominal carcinoma	9.61
24	M	Abdominal carcinoma	10.51
25	M	Carcinoma of stomach	11.59
26	F	Carcinoma of stomach	9.04
27	M	Carcinoma of esophagus	7.61
28	M	Carcinoma of rectum	8.28
29	M	Carcinoma of bladder	6.44
30	M	Carcinoma of bladder	10.89
31	F	Carcinoma of uterus	10.40
32	M	Carcinoma of vocal cord	10.04
33	M	Sarcoma of thymus	10.74
34	F	Sarcoma of uterus	9.68

* Abdominal carcinoma indicates generalized peritoneal metastases, primary tumor not located. All diagnoses confirmed by histological examination.

TABLE 2

	SEX	DIAGNOSIS	CALCIUM PER 100 CC.
			mgm.
1	M	Thromboangiitis obliterans	10.51
2	M	Thromboangiitis obliterans	5.41
3	M	Thromboangiitis obliterans	9.87
4	M	Thromboangiitis obliterans	8.06
5	M	Thromboangiitis obliterans	10.80
6	M	Thromboangiitis obliterans	11.51
7	M	Thromboangiitis obliterans	12.34
8	M	Thromboangiitis obliterans	9.90
9	M	Thromboangiitis obliterans	8.65
10	M	Thromboangiitis obliterans	11.97
11	M	Thromboangiitis obliterans	12.21
12	M	Multiple exostoses	10.58
13	F	Cystic goiter	12.51
14	M	Congenital cystic kidney	7.65
15	F	Cystadenoma of ovary	10.29
16	F	Cystadenoma of ovary	8.65
17	F	Papilloma of bladder	11.21
18	F	Pulmonary tuberculosis	14.31
19	M	Healed pulmonary tuberculosis	8.73
20	M	Cholecystitis	9.54
21	M	Cholecystitis	11.12
22	M	Pneumonia (ante mortem)	8.23
23	M	Abscess of lung	11.43
24	F	Chronic appendicitis	8.20
25	F	Syphilis	10.54
26	M	Chronic nephritis	9.51
27	M	Chronic nephritis	7.80
28	M	Chronic nephritis	11.40
29	F	Eclampsia	4.29
30	M	Acromegaly	7.70
31	F	Acromegaly	13.33
32	F	Exophthalmic goiter	10.66
33	M	Diabetes	12.68
34	M	Diabetes	9.58
35	M	Pernicious anemia?	8.69
36	F	Cerebral degeneration, arteriosclerosis	9.22
37	M	Gastroptosis	11.15
38	F	Neurosis	11.38
39	M	Neurosis	8.26
40	F	Bronchial asthma	8.96
41	F	Obscure abdominal condition	10.51
42	M	Tumor of lung?	9.89
43	M	Tetany	6.11

neoplasm is not in evidence. In six benign tumors (table 2) the results were similar to those obtained in malignant disease; they varied from 7.65 to 12.51 mgm., and averaged 10.14 mgm.

Table 2 also records the calcium figures of eleven cases of thromboangiitis obliterans, as well as the clinical diagnoses and blood calcium values of twenty-six miscellaneous cases, in which no sign of malignant disease was manifest. In the miscellaneous material, no one disease is represented by a number of cases sufficiently large to permit of definite conclusions regarding changes in the calcium concentration that may occur; the figures are presented, however, because additions to the blood calcium data available at present, seem indicated.

For thromboangiitis obliterans, the average calcium concentration in the blood is again within normal limits (10.11 mgm. per 100 cc.), while the variations in individual cases (5.41 to 12.34 mgm.) would tend to show that a relationship between this disease and calcium metabolism does not exist.

Regarding individual cases, it may be of interest to note that only 4.29 mgm. of calcium were found in a case of eclampsia, and that 6.11 mgm. occurred in a typical case of tetany, the latter figure in accord with those observed by Howland and Marriott (6). In chronic nephritis our results agree with those of Halverson, Mohler, and Bergeim (7), a low figure of 7.8 mgm. occurring in a case with retention, while two of a milder type presented concentrations within normal limits. On the other hand, 14.31 mgm. occurring in one of our tuberculous patients, is rather high as compared to the figures obtained by Halverson, Mohler, and Bergeim in their study of thirty cases of tuberculosis.

SUMMARY AND CONCLUSIONS

The blood plasma calcium content was determined in thirty-four cases of malignant disease, in six of benign tumors, in eleven of thromboangiitis obliterans, and in twenty-six miscellaneous cases.

In cancer the average calcium values were within the figures generally accepted as normal, and no characteristic concentration accompanied any given type or location of neoplasm. In benign tumors the results were similar.

The average calcium figure for thromboangiitis was within normal limits, while the variations in individual cases would indicate that calcium metabolism has no connection with this disease.

In accordance with the results of other authors, low calcium values were obtained in severe nephritis, in eclampsia, and in tetany.

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PRIMARY SPONTANEOUS TUMORS OF THE OVARY IN MICE

STUDIES ON THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE

Fourteenth Communication

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Throughout the animal kingdom the solid ovarian tumors seem to be infrequent, as they also are, relatively, among human tumors. Cystic tumors are described occasionally, but apparently less frequently in the lower animals than in man. Even among dogs, with their high incidence of tumor growth, ovarian tumors are rare according to the evidence furnished by literature dealing with canine neoplasms.[1] In Sticker's (2) compilation of tumors in domestic animals, of 766 tumors in dogs but 3 were in the ovary. As to other animals of Sticker's series, in 509 cases of tumors in horses, 4 were in the ovary; of 110 in cattle, 6 were ovarian; there was 1 ovarian tumor among 23 tumors in cats, and none at all among sheep, goats, and swine. Kimura (3) has reported 142 cases of tumors in horses, among which were no ovarian tumors, although there were 49 in the testicle. Other evidence supports the figures of Sticker in indicating that cows have ovarian tumors more often than other species. Trotter (4) reported 305 bovine tumors observed in the Glasgow slaughter house, of which 5 were in the ovary (4 carcinomas and

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[1] Goodpasture (1) notes the occurrence of small hyperplastic areas in the senile ovaries of old dogs, but even these do not seem to be of very frequent occurrence in proportion to the high incidence of proliferative changes noted by him in other tissues.

1 sarcoma). These were all large lobulated solid tumors, and no metastases were observed in any. Leo Loeb (5) has described a tumor arising in the ovary of a six-months-old calf, composed chiefly of cells resembling luteum tissue.

Among the numerous instances of tumors in wild rats reported by McCoy (6), Woolley and Wherry (7), and Beatti (8) there is no case of ovarian growth; nor have we found reports of any cases occurring in domesticated rats.[2]

Wolff (9) mentions 2 cases of ovarian tumors in cats: One a primary carcinoma in the ovary of a thirteen-year old cat with metastasis in the liver, reported by Kitt; the other a sarcoma of the ovary and pelvis reported by Stroud.

Wild animals are also unlikely to have ovarian tumors. Fox (10), in his extensive studies of tumors in wild animals, has described no cases whatever of ovarian tumors among the mammalia.

Only in birds do ovarian tumors seem to be relatively frequent. Bürger (11) found, among 852 fowls autopsied at the Leipzig veterinary institute, 12 tumors, of which 7 were in the ovary, 4 being sarcomas and 3 carcinomas; 2 of the sarcomas and 2 of the carcinomas had produced metastases. In their review of the literature on tumors in birds, Joest and Ernesti (12) found 112 cases reported and added 50 more. Of these 162 cases, 21 were primary carcinoma of the ovary, commonly with extensive peritoneal and visceral metastasis. There was also one case of ovarian sarcoma.

An ovarian tumor has been described in a wild turkey (*Meleagris gallapavo*) by Fox (10), as follows: The growth was "about the size of 3 English walnuts placed in triangular position." It consisted of 3 subdivisions, covered with papillomatous prominences like a hydatid mole. Microscopically it was a papillary cystadenoma.

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[2] While this article was in press there appeared under the title "Carcinosarcoma de rata blanca," an article by Dr. A. H. Roffo (Revista del Instituto Bacteriologico, Buenos Aires, 1919, ii, 283), reporting the finding of a large tumor in the ovary of a white rat. This seems, from the illustration and description, to have been a papillary cystadenoma with areas of more compact cell growth, some of which are interpreted as carcinoma and some as sarcoma.

Cold-blooded animals also have furnished occasional cases of ovarian tumor. Bland-Sutton (13) has reported a case of tumor of the ovaries of a python, with growths also in the lungs, liver, and peritoneum; he believed the ovarian growth to have been primary but without conclusive evidence. Plehn (14) has described tumors arising in the ovary of an old grass frog (*Rana esculenta*) through growth of primitive ova cells without differentiation. There were numerous nodules, from millet seed to cherry size, apparently benign in character although resembling a malignant tumor in histological structure. A cystic tumor of the ovary has been described in a fish, the ling (*Molva molva*) by Johnstone (15).

In mice, also, ovarian tumors are not common, but we have found mention of 8 cases in the literature. The first 2 cases were described by Jobling (16). One was bilateral, each ovary being eight times the normal size. Both ovaries showed the same structure, which is described as follows:

> A great increase in epithelial cells, which formed solid masses, somewhat compressed and elongated into spindle cells and cysts of different sizes, usually small and more or less occupied by the papillary outgrowths from their walls. These outgrowths developed from narrow or somewhat thickened pedicles and spread out into a fanlike structure. . . . . The more solid portions were at one time cystic but the cysts became occluded by the ingrowth of papillae. Acini possessing a distinctly granular [sic., glandular?] form and arrangement also occurred. Mitosis was rare, direct division more frequent. Hemorrhage had taken place into some of the cysts. Mallory stain showed a delicate connective-tissue basement membrane surrounding the small cysts and the more solid areas, the latter being filled with epithelial cells of a granular quality resembling somewhat the lutein cells.

In the other case the growth was unilateral, there being numerous large cysts, separated by smaller ones and by the tubules of the ovary, lined by high columnar epithelium without cilia. Between the cysts the tissue was composed largely of smooth muscle fibers, resembling in places a leiomyoma.

Tyzzer (17) has reported 4 cases. 1. This tumor was bilateral, the ovaries being replaced by an irregularly glandular epithelial growth, in places with flattened epithelial cells intimately mingled with the connective tissue; in others the glands contained thick papillary processes of epithelium without central connective tissue core protruding into the lumen, the epithelium being in places high columnar, in others merely a fused mass. There were no mitotic figures, but the presence of masses of epithelium in the lymphatics suggested malignancy. 2. In a mouse with a renal tumor resembling a hypernephroma, one ovary was replaced by an irregular gland-like structure similar to the above. 3. A mouse of a series bred for heredity studies, which died from a lung tumor, had the right ovary replaced by a mass the size of a large pea, composed of irregular epithelium having in places a partially glandular structure, without tendency to form spaces. 4. A mouse from the same family as (3), had both ovaries replaced by masses of translucent whitish tissue, 5 by 3 by 3 mm. This also had the structure of a papillary adenoma. Tyzzer notes that these growths differ from the ordinary types of ovarian tumors seen in human beings, in that the epithelium is more or less glandular with but little tendency to form cysts. The epithelium resembles the peritoneal mesothelium found at the attachment of the ovary.

Haaland (18) found 2 ovarian tumors among 353 primary tumors observed in 288 mice, of which 325 were mammary gland growths. 1. A mouse that had a sarcoma arising in a scar also had a tumor the size of a hazel nut in the left ovary. The ovarian tumor was composed of large alveoli with a peripheral layer of low columnar epithelial cells, the lumen filled with round cells and sometimes exhibiting spaces filled with serous fluid. This tissue was transplanted into 120 normal mice, but no growths resulted. 2. A mouse that had been operated for mammary carcinoma with recurrence. A tumor "as large as a pea" replaced the right ovary, and in structure was a papillary adenocarcinoma, probably primary.

Of these 8 recorded cases of ovarian tumors in mice there were 3 cases of bilateral tumors, although in no case was there metas-

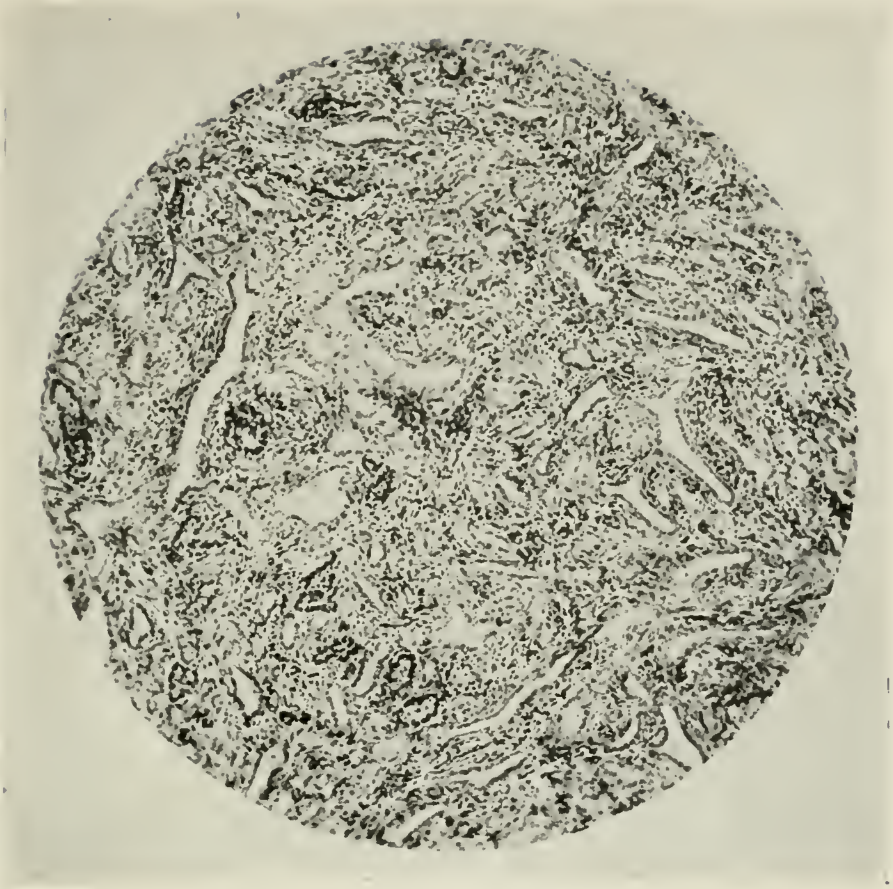

Fig. 1. Solid Tubular Adenoma

This is the most usual type of benign tumor of the ovary in mice. Much of what seems to be stroma between the tubules is really composed of compressed epithelial cells. Mouse 12760. × 110.

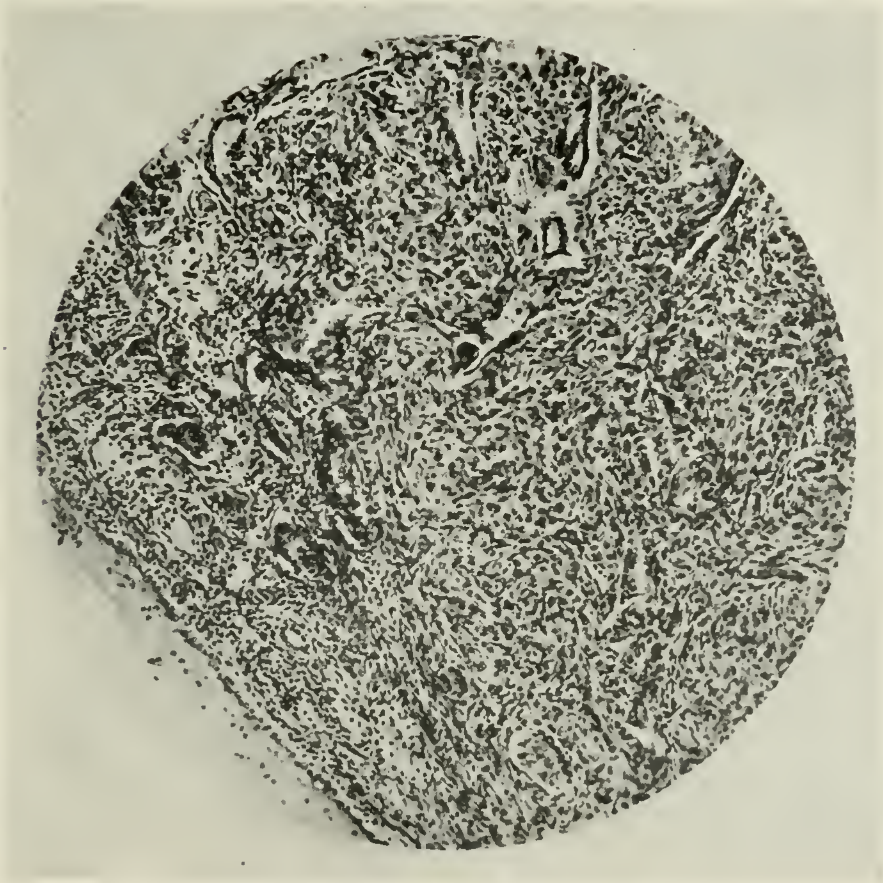

Fig. 2. Solid Tubular Adenoma

Showing a larger proportion of compressed epithelial masses. × 110 tasis or other posirive evidence of malignancy. Four of the eight mice had tumots elsewhere than in the ovaries. All but one of the tumors were of similar structure, consisting of atypical glandular or alveolar formations with a tendency to epithelial proliferation into the lumen to form solid plugs or papillae—apparently best described as solid, atypical papillary adenoma.

Among 22,000 mice of the Slye stock that have come to autopsy have been found 46 with solid tumors that seemed to be primary in the ovary. This may be compared with 28 testicle tumors in 19,000 autopsies, 160 lung tumors in 6,000 autopsies, 87 sarcomas in 12,000 autopsies, and 4 cancers of the stomach in 16,500 autopsies.[3] These figures indicate that, as with other animals, solid ovarian tumors are not common in mice. There have been observed simple cystic conditions in the ovaries of but a small number of mice, and ovarian cysts are apparently rather infrequent. The strikingly large cysts seen in women have never been observed. In only a few of the

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[3] We would again emphasize the character of the material from which these tumors have been obtained, and the conditions under which the growths have developed. The 22,000 mice are all the descendants of a limited and carefully selected stock, bred together according to definite plans designed to give evidence as to the influence of heredity upon the incidence of spontaneous tumors in mice, and, hence, including strains of highly cancerous ancestry and strains with ancestry free from cancer. They represent strains in which cancer is very common, strains in which it never occurs, and strains of intermediate character. The influence of heredity on the incidence of ovarian tumors will be considered elsewhere, and we mention these facts here to indicate the character of the material in this respect. It must also be emphasized that none of these mice has been subjected to any artificial influences that might modify its life. In no case is a spontaneous tumor used for inoculation, or operated upon, and no mouse born in this laboratory is ever used for any experimental work whatever. From the moment of its birth every effort is directed to the one object of permitting each mouse to reach a maximum age. Long experience and great care have made it possible to limit to a large extent the epidemic infections that constantly threaten such large colonies of mice under even the best of conditions. Of especial importance is the fact that every mouse that dies is submitted to a careful post-mortem examination, no matter whether it dies in infancy, from an accident, or from any other obvious cause; and every suspicious area is submitted to microscopic examination by three people or more. Were it not that every dead mouse is thus thoroughly investigated, and that the average age at death is, for a mouse community, very high, we should not have nearly so much material to describe.

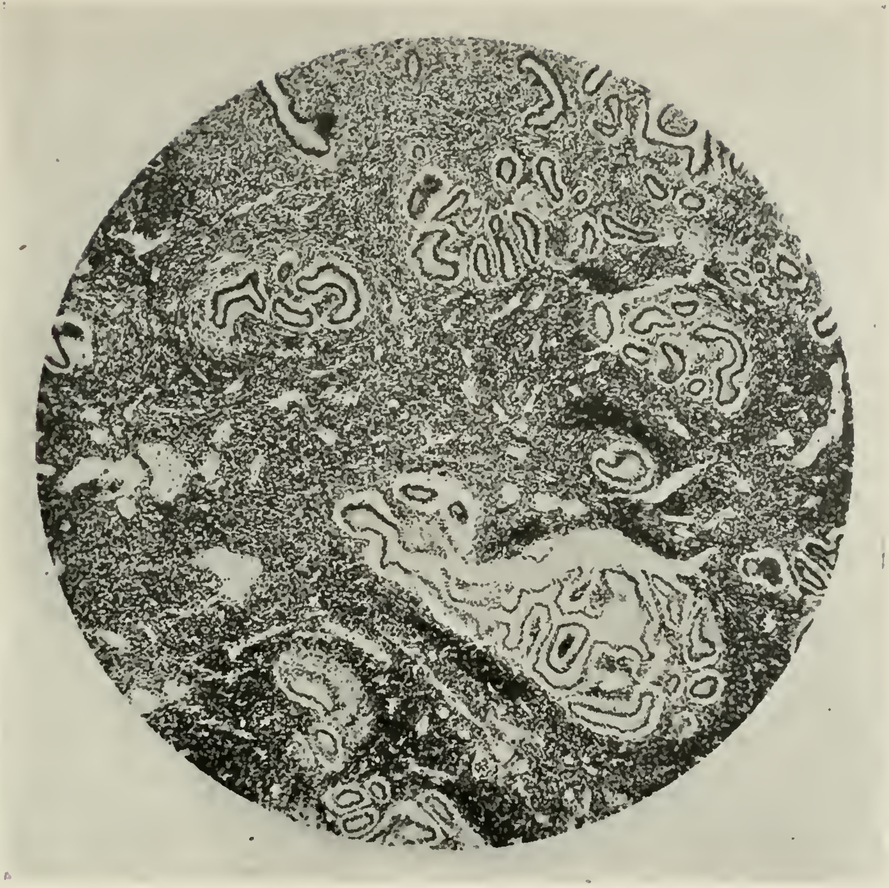

FIG. 3. SOLID TUBULAR ADENOMA

In this growth there is an unusually large proportion of ovarian stroma type. × 76.

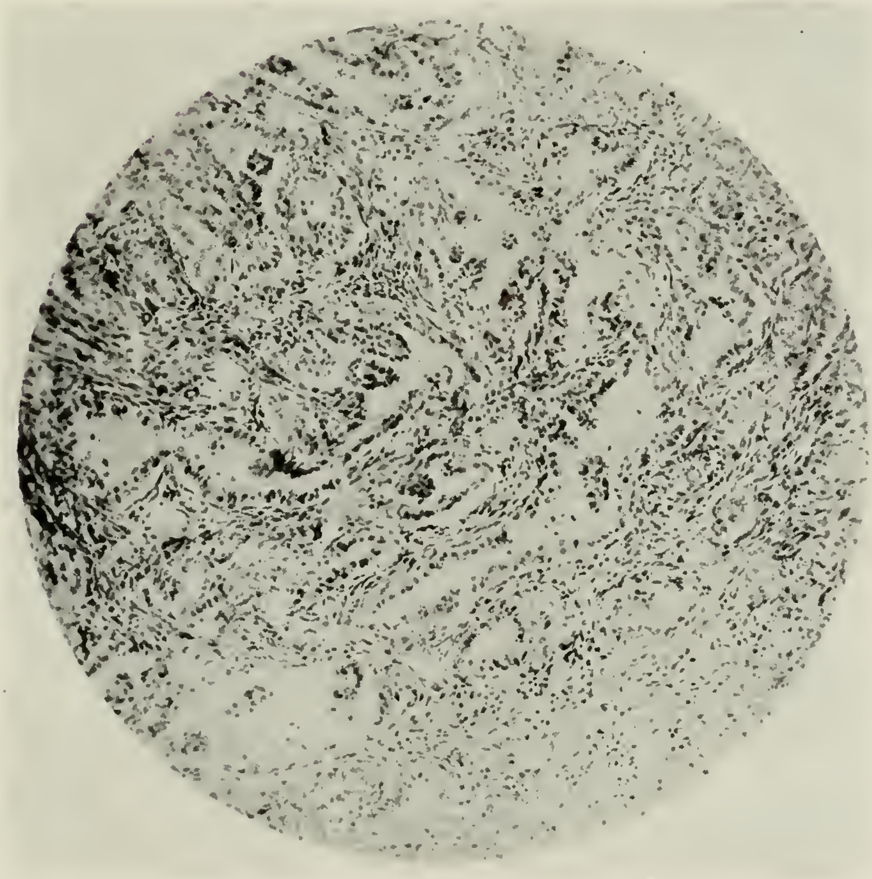

FIG. 4. PAPILLARY PORTION OF A GROWTH THAT ELSEWHERE IS A SOLID TUBULAR ADENOMA, AS IN 1 AND 2

Small areas thus disclosing an existing tendency to papillary structure are not infrequently found in the ordinary solid ovarian tumors of mice. Mouse 2205. × 110.

ovarian cysts that we have examined have small cystic papillomatous areas been found. Therefore, mouse ovarian tumors present a marked preponderance of solid adenomatous growth, as compared with the proportion of cystic ovarian enlargements in women. We have eliminated from our consideration the simple cysts, as probably not examples of neoplastic growth, with the exception of those cysts that result from secretion by a cystadenoma.

Most of our tumors seem to be benign in character, although we have found a few examples of undoubted malignant tumors primary in the ovary. In all, 38 mice have exhibited tumors that may be classified as solid benign ovarian tumors, and there was one typical papillomatous cystoma. As 19 of these had bilateral tumors there are 58 ovarian tumors occurring in a stock of mice that have yielded over three thousand primary spontaneous tumors of other tissues, chiefly the mammary gland.

BENIGN OVARIAN TUMORS

In structure these tumors vary considerably, although most of them correspond closely to the descriptions given by Jobling, Tyzzer, and Haaland, and may be most appropriately designated as solid alveolar adenoma. Tendency to cyst formation is exceptional, in contrast to the adenomas of the human ovary, and the same is true of papillary types of growth, which are rarely exhibited. In general these solid adenomas of the mouse ovary exhibit a growth apparently under great pressure, with crowding of the cells so that it is usually difficult to differentiate readily between stroma cells and flattened epithelial cells (see figs. 1 and 2). Probably this crowding of the growth accounts for the absence of papillary tendency, for often a small area may be found where there is less pressure or where part of the tissue has been destroyed by hemorrhage or necrosis, in which a distinct tendency to papillary structure is seen (see fig. 4). We have only one case (12111) in which the structure corresponds at all closely with the human papillary cystoma (fig. 7).

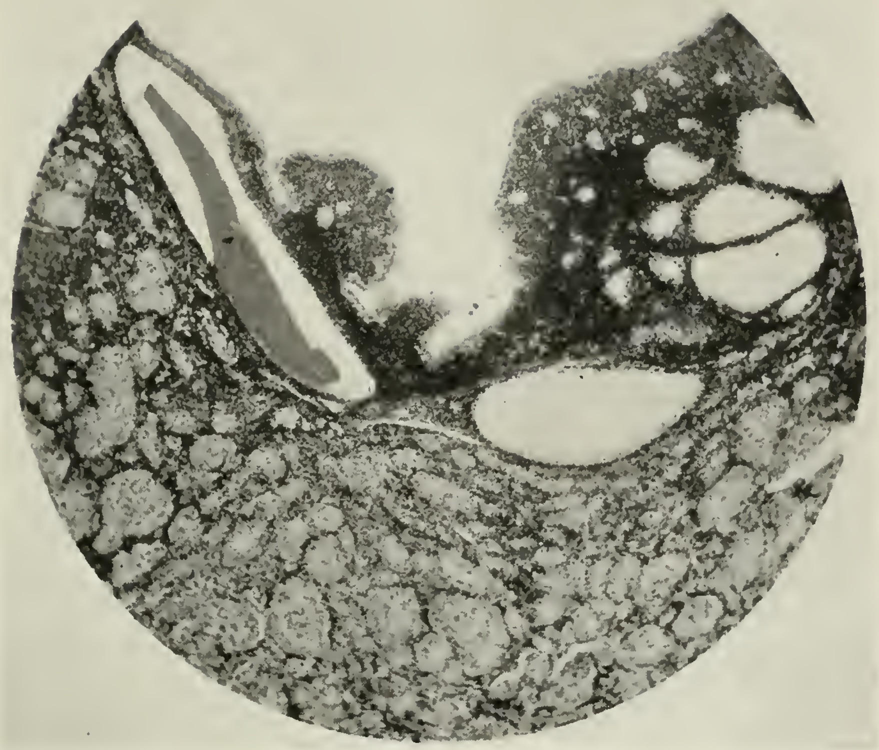

FIG. 5. OVARIAN TUMOR EXHIBITING CYSTADENOMA TYPE OF GROWTH IN ONE PORTION, WHILE ANOTHER PART SHOWS A SOLID ALVEOLAR CHARACTER RESEMBLING PRIMITIVE FOLLICLES
Mouse 6991. × 50

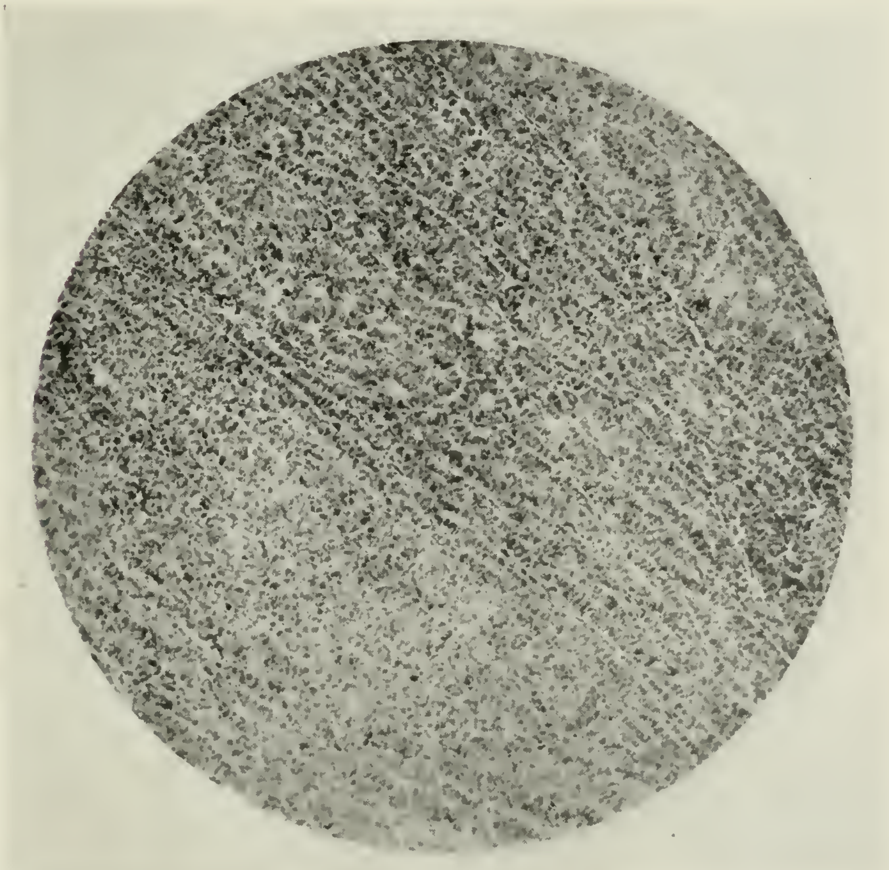

FIG. 6. SOLID TUBULAR ADENOMA

Consists throughout of a solid compressed mass of epithelial tubules, with a minimum of stroma. Mouse 20207. × 110.

Except for the few malignant growths in this series, nearly all the tumors seem to be fundamentally similar growths, yet differing considerably from one another according to the degree of differentiation permitted by the pressure under which the cells are growing. The usual character may be described as follows: The affected ovary is as a rule, uniformly enlarged, commonly to a diameter of 3 to 10 mm., white, firm, and often with a lobulated surface. In half of the cases the growth is bilateral, (19 of 38), usually one of the ovaries being considerably larger than the other.[4] The capsule is distinct but there may be adhesions to the adjacent tissues. If cysts are present they usually contain a clear watery fluid, but may have a blood-stained content. In most cases the benign ovarian adenomas have caused no apparent trouble; usually they are autopsy findings in mice dying from some unrelated condition. Sometimes fatal hemorrhage has occurred from ovarian tumors. In passing, it may be mentioned that large hematomas often form in the ovaries of mice without neoplasms, the blood usually forming strikingly laminated clots.

Microscopically the enlargement is usually caused by a compact growth of cells of two types, low cuboidal epithelial cells and spindle-shaped cells resembling those of normal ovarian stroma, but which often are definitely, in part at least, composed of compressed epithelial cells (see figs. 1 and 2). The cuboidal cells may form tubules, or solid plugs resembling the "Pflüger's tubes," or solid alveolar masses which may resemble primitive follicles, (see fig. 5), or occasionally alveoli into which papillary or fan-shaped outgrowths of the lining of the epithelium are crowded.[5] Most of the tumors show more or less of each of these types of growth, one type generally predominating. Sometimes one part of a section is composed solely of one type and another part of another type. Sometimes these various structures are

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[4] This corresponds well with the figures given for human solid ovarian tumors by Massabuau and Etienne (19), who found that of 250 such tumors 43 per cent were bilateral. Of the eight cases of ovarian tumors in mice reported by Jobling, Tyzzer, and Haaland, three were bilateral.

[5] Such a type of growth is shown by Tyzzer (17) in his figure 24.

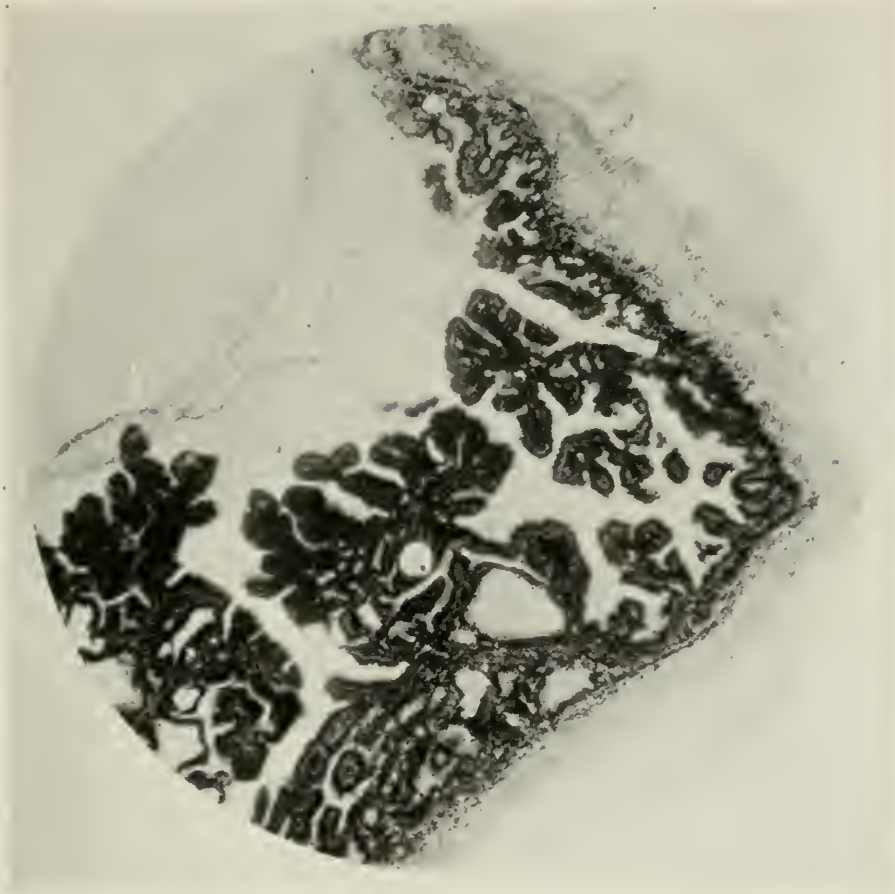

FIG. 7. PAPILLARY CYSTADENOMA

The only specimen in our series typically reproducing this type common in the human ovary. Mouse 12111. × 45.

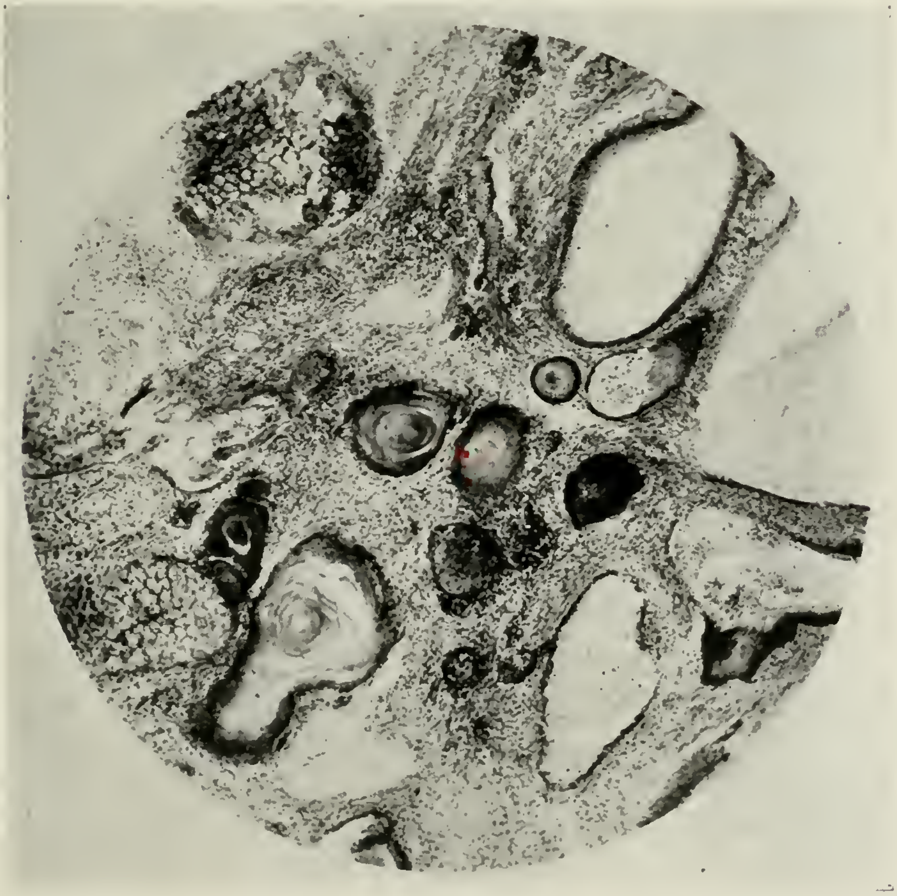

FIG. 8. SOLID TERATOMA

Shows in this field islands of cartilage, epithelial plugs with hornified epithelium, cysts lined with squamous and with columnar epithelium, and varying types of stroma elements. Mouse 9278. × 60.

limited by a distinct basement membrane of true stroma cells, but often there seems to be a false stroma of crowded, spindle-shaped epithelial cells without sharp demarcation from the true stroma cells that may be present. Usually the total amount of stroma is small, most of the growth being composed of epithelial cells, and this stroma is of the cellular ovarian type and not ordinary fibrous tissue. Not infrequently, however, the stroma elements form the bulk of the growth (fig. 3). Generally the capsule is formed by compressed ovarian tissue, in which ova or follicles are rarely seen. Blood vessels are usually scanty; but, nevertheless, necrosis or other retrogressive change is seldom found. Mitosis is very rarely observed, nor are forms suggestive of amitotic division common. The epithelium resembles the germinal epithelium and occasionally the surface of the tumor is covered with this cuboidal epithelium which dips down at intervals to divide the growth deeply into lobules.

As variations from the structure described above we have in a few instances the formation of several small cysts with lining of flattened epithelium. In a few also the structure suggests a papilloma with all spaces obliterated by pressure or from lack of secretion by the surface epithelium. If the growth is of tubular character the pressure usually causes the lumens to resemble narrow slits (figs. 2 and 6). In some instances the alveolar structure is filled with flattened cells consisting chiefly of deeply staining nuclei, producing a growth resembling the adenomatous growths often found in the human vermiform appendix. In only one instance have we a true fibroadenoma (12922) in which a large part of the tumor is composed of definite fibrous stroma with abundance of collagenous material, rather than the cellular ovarian type of stroma. We have not identified any of our tumors as of lutein cell structure, nor have we observed fibromas or myofibromas in the ovary.

MALIGNANT OVARIAN TUMORS

We have found the following tumors that seem to be unquestionably primary malignant tumors arising in the ovary.

6487. The left ovary was 10 mm. in diameter, nearly spherical, partly white and fleshy, partly cystic. There were no

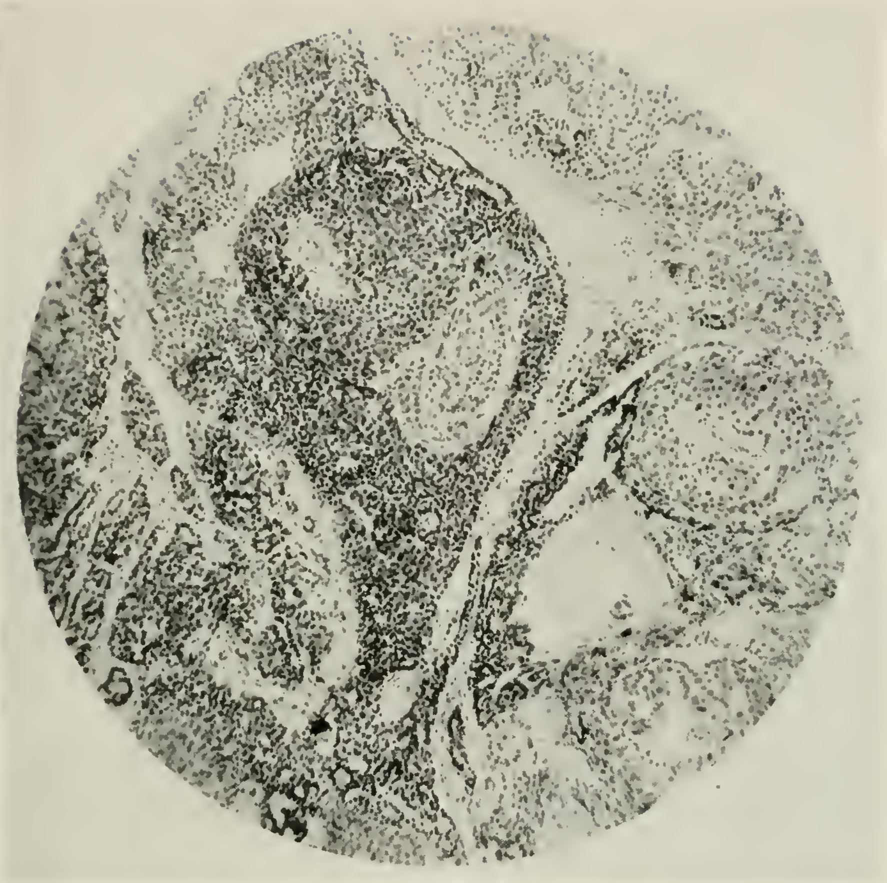

Fig. 9. Malignant Epithelial Tumor of Ovary

Showing both tubular and alveolar types of growth. Mouse 6487. × 100

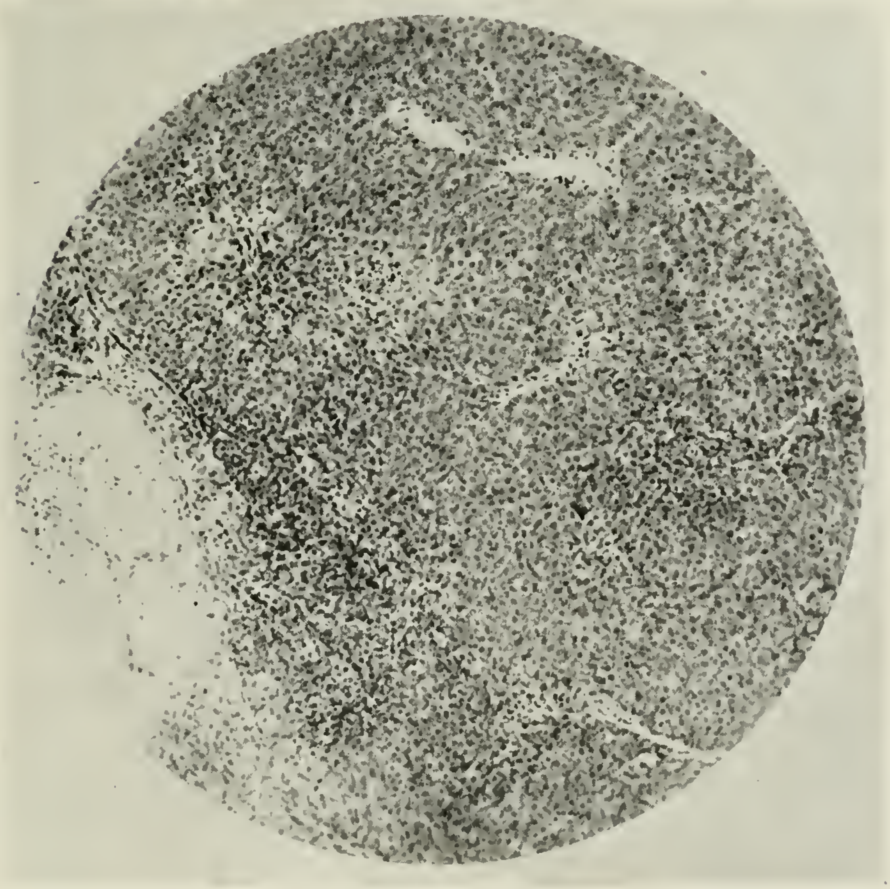

Fig. 10. Malignant Epithelial Tumor of Ovary

Showing solid type of growth of large cells without any well defined arrangement. Similar structure is also shown by tumors of testicle and adrenal. Mouse 12552. × 110.

adhesions and no evidence of metastasis. The mammary gland exhibited a large hemorrhagic carcinoma and also a minute early carcinomatous nodule, both of these growths being typically and unquestionably primary carcinomas of the mammary gland; structurally they were entirely distinct from the ovarian growth. About one half of the latter was composed of a cavity filled with a protein-rich fluid, and with walls formed by an irregular border of tumor tissue. Apparently this cyst was formed by degeneration of tumor tissue, as it had no proper cyst wall, although on the side towards the tumor there are some papillary outgrowths into the cavity. The solid part of the tumor consists of epithelial cells arranged mostly in large irregular alveoli, often exhibiting central necrosis, and occasionally atypical tubules are seen (fig. 9). The stroma is scanty and of ordinary connective tissue type for the most part, although these are areas with abundant elongated cells. The cells are large with a clear cytoplasm, well defined borders, and oval nuclei which are partly solid and partly slightly vesicular. Occasional mitotic figures are seen. Although there are no metastases, the histological structure of this tumor is such that it must be considered to be malignant, an alveolar carcinoma of the ovary.

12552. The left ovary measured 25 by 18 by 18 mm., nodular, with many distended blood-vessels coursing over it. It was not adherent to adjacent tissue and no metastatic growths were found. In the mammary gland were two hemorrhagic carcinomas, entirely different in structure from the ovarian tumor. Microscopically the ovarian tumor is composed of large irregular alveoli filled with large cells with a moderate amount of cytoplasm and a large pale nucleus, with very little stroma (fig. 10). Several mitotic figures were found. The original ovarian capsule surrounds the growth, but no recognizable ovarian tissue remains. Areas of necrosis and hemorrhage are numerous. This tumor closely resembles 6487 but exhibits a greater degree of malignancy.

6801. The left ovary was converted into a hemorrhagic nodular mass, 18 by 15 by 10 mm. The right ovary was normal.

A mass of tumor tissue lay anterior to the left kidney, about the size of the kidney itself. There was a large carcinoma of the mammary gland arising in the left flank, and producing metastases in the lung, as shown by microscopic examination.

The large ovarian tumor consists of about equal parts of extravasations of blood or large dilated blood channels and of tumor tissue. Nothing of the original tissue remains, except that in the capsule appear a few cells suggestive of ovarian stroma elements. The tumor has no well defined structure, consisting of large solid masses of cells which occasionally are formed into trabeculae by blood channels, but otherwise show no tendency toward grouping of any kind. The cells are characterized by a considerable amount of cytoplasm which is usually homogeneous and often with well defined cell borders. The nuclei are round, sometimes solid and sometimes vesicular. Where the cells are largest they resemble slightly liver cell types. There is practically no stroma besides the blood vessels, which are scanty except for the large channels in the tumor without vessel walls. The retroperitoneal mass presents quite the same structure. Mitotic figures are not seen. A few multinucleated cells are found. The general character of the growth resembles that of other malignant tumors found in the adrenal and testicle.

14099. The left ovary is about three times the normal size, and of a red color. The uterus is distended with a milky fluid. In the upper lobe of the right lung is a firm nodule, 2 mm. in diameter, which proved to be a simple papillary adenoma, apparently benign but with a projection growing into a blood-vessel. There were no changes of significance in the other organs. Microscopically the ovary is found to be almost entirely replaced by a mass of large cells with no particular arrangement, which invade the remaining recognizable ovarian tissue, and in which appear epithelial structures that resemble overgrown Pflüger's tubules. The nuclei are deeply stained and irregular in size. In the perirenal tissue is a small growth of similar character.

It is interesting to observe that the malignant types of tumors arising from the ovary, testicle, and adrenal that we have studied exhibit such a similar histological picture, and one quite dissimilar from tumors arising in other tissues. There can be little doubt that they all represent reversions to the primitive embryonal tissues of the urogenital anlage, and are probably best designated by Adami's term, mesothelioma. This similarity of structure makes it difficult to determine the origin of a tumor involving both the ovary and the adrenal, as in the following case.

12307. The abdominal cavity shows several nodules whose exact origin is difficult to determine as the mate has partly devoured the body. The right ovary is, however, easily distinguished. It measures 18 by 12 by 12 mm. What seems to be the left ovary is 10 by 8 by 6 mm. There are 8 other similar nodules in the abdominal cavity, one being in the position of the left adrenal, measuring 10 by 8 by 8 mm. The other nodules are apparently in the mesentery. One lobe of the liver is converted into a tumor nodule 14 by 10 by 18 mm., irregular and lumpy in outline, pink in color.

The tumor tissue shows everywhere the same structure, consisting of irregular alveoli composed of large cells with abundant cytoplasm with well defined borders and deeply staining nuclei. Mitotic figures are numerous. The character is that usual to mesothelial growths. The ovary cannot be positively identified, but one mass exhibited in the capsule structure suggests compressed ovarian tissue with degenerated ovum. In all respects this tumor is identical with the malignant ovarian tumors just described.

It seems probable that this tumor arose in the ovary which exhibited the largest growth, but it is not possible to exclude the adrenal as the primary site.

Still more difficult to locate is the primary growth in the following case.

12876. The left kidney contains a mass of pink, fleshy tissue, 18 by 14 by 14 mm. The right kidney, which is slightly enlarged, contains no tumor mass. The right ovary consists of a pinkish tissue resembling that in the kidney, and measures 12 by 8 by 8 mm. In the mesentery is a similar, slightly paler mass, 16 by 8 by 8 mm. The retroperitoneal and subcutaneous glands are not enlarged and no nodules are found in the lungs.

Microscopically the tumor tissue is alike in all three places, consisting of a diffuse infiltrating growth of large round cells, which also invade the connective tissues about the kidney and ovary. It does not at all resemble the typical ovarian tumors, being apparently a round-cell sarcoma. We have no way of telling which of the three tumors was primary. The next case presents similar difficulties.

26. This mouse had a tumor mass about 8 by 10 mm. in the upper portion of the liver, with other smaller nodules near it. A similar small nodule was found in the right kidney. The right ovary was enlarged to two-thirds the size of a kidney, and was solid. Microscopically all these growths are composed of round cells, apparently a round-cell sarcoma. It is impossible to say which growth was primary.

We have seen few instances of secondary tumors occurring in the ovary. The Krukenberg tumor is not found because mice do not have gastric or other abdominal carcinomas, except most rarely (20).

In leukemia and pseudoleukemia, infiltration of the ovary with lymphoid elements is common, and often very striking. In our collection there has been no case observed of secondary carcinoma of the ovary, which is not surprising in view of the relatively slight tendency of mouse carcinomas to produce metastasis elsewhere than in the lungs. The following examples of secondary sarcoma have been noted:

12058. A bilateral sarcoma of the uterus, round-cell in type, with metastasis in the right kidney. The left ovary was 10 mm. in diameter and showed complete replacement by sarcoma tissue. As near as we can determine the growth in this case arose in the uterus, and invaded the ovary by infiltration.

19061. A spindle-cell sarcoma growth infiltrated the retroperitoneal tissues extensively, including the pancreas, also with metastasis in the liver. Apparently primary in either the retroperitoneal tissues or in the mesentery. The uterus and both ovaries were infiltrated by the same tissue.

To summarize, we have found four malignant growths that seem to be certainly primary in the ovary. Each of these exhibited the structure common to malignant tumors arising in the sex glands and adrenal. One exhibited retroperitoneal metastasis near the kidney. Another tumor of similar histological type also involved the adrenal, and produced numerous metastases; in this case it was not possible to determine whether the growth was primary in the ovaries or in the adrenal.

There were two round cell sarcomas involving the ovary as well as other organs, which might have been primary in the ovary, but the evidence did not permit of deciding this. There were two other cases in which the sarcomatous invasion of the ovary seemed to be unquestionably secondary. No secondary carcinomas were observed in the ovary.

TERATOMA OF THE OVARY

From the accounts of animal tumors in the literature it would seem that teratomas are extremely infrequent in the lower mammals. In reviewing the literature on the occurrence of tumors of the ovary and testicle, in the lower animals the chief sites of teratomatous growths, we have found mention of but one case. That was described by Winokuroff (21) as a teratoma of the testicle in a chicken, the growth exhibiting cartilage, bone, striated muscle, squamous epithelium, and cysts. Our own experience supports the view that the lower animals rarely exhibit teratomas, for in the 22,000 mice here considered containing over three thousand spontaneous primary tumors, we have observed but one teratoma. This arose in the ovary, and is a typical example of solid teratoma as shown by the following description:

9278. Death resulted from pulmonary infection. There were no other changes of importance except in the left ovary, which measured 20 by 18 mm., and yielded an exudate from the cut surface. Microscopically the growth has a delicate but distinct capsule in one portion of which there still remains a trace of the original ovarian tissue with one follicle containing an ovum. Except for this the entire section shows a mass of tissues of all sorts thrown together in an entirely disordered manner (fig. 8). There are numerous small cavities, some of which are lined with squamous epithelium containing chiefly desquamated cells and some polynuclear leukocytes. Occasional solid plugs of squamous epithelium also occur. In places these are branching and occasionally a basal-cell type of growth is seen. No true hair follicles are recognized although occasionally the epithelial downgrowth suggests these structures. No sebaceous glands are found. Most of the squamous-cell structures are grouped together in localized areas. There are also spaces lined with columnar or flattened epithelium, sometimes with a content resembling a diluted mucin. Spaces are found in which part of the lumen is lined with stratified epithelium and part by cuboidal or columnar epithelium. Sometimes these tubules have a well defined coat of non-striated muscle fiber. Small islands of cartilage are abundant, usually having no definite relation to other structures. No bone is seen. Some of the cavities contain old extravasations of blood in varying stages of disintegration. In one place there is a mass of heavy brown pigment near which are collections of small round cells; the whole appearance suggests that possibly this area represents undeveloped retinal tissue. The stroma in general consists of a loose fibrous tissue with abundant cells. There are also many cells with much more cytoplasm than ordinary connective tissue cells, this cytoplasm having a slight basophilic tendency. No striated muscle is found or definite organ tissues but there are many accumulations of cells which are not stroma cells and which presumably are undifferentiated cells of special tissues.

Since this analysis of 22,000 autopsies was made, another instance of teratoma of the ovary has been observed, so that we now have found two teratomas among 25,000 autopsied mice. The chief features of this second case are as follows:

24172. Mouse, eleven months old, died apparently from acute pulmonic infection following delivery, but when found post-mortem changes were too far advanced to be certain of the diagnosis. The puerperal uterus showed no gross evidence of infection. There were no tumors except that involving the right ovary, which measures 30 x 15 x 15 mm., and is nodular, encapsulated, but not adherent. The mass is soft in consistency and heterogeneous in appearance. There is no exudate or cyst formation on the cut surface.

Microsopically the growth is found to be composed of many different sorts of tissue elements, but unfortunately a large part has undergone so much necrosis and post-mortem change that the structures cannot be well studied. In the portions that do stain the elements are extremely varied and without any particular relation to one another. The greater part of the tissue elements cannot be identified as to their origin or character, being merely masses of cells with small nuclei and considerable cytoplasm. Conspicuous are the plugs of squamous epithelium, with masses of hornified material in the center, but recognizable skin, hair follicles, or sebaceous glands are not found. Tubules lined with columnar epithelium are also occasionally found. There is unusually little tendency to form epithelial-lined cavities or cysts. Numerous areas of mucoid degeneration are present, but goblet cells are not present in these areas, nor can the cellular origin of the mucin be determined. There are a few small islands of bone tissue, but only a few minute groups of cartilage cells. Nonstriated muscle fibers are abundant, usually in distinct bands, but striated muscle fibers are not found. A very little fatty areolar tissue is present. Except for fibrous tissue and blood vessels these are all the tissue elements that can be identified, although some areas resemble liver cells, and others simulate developing nervous tissues. Nothing suggestive of malignancy is found. The diagnosis is clearly solid teratoma of the ovary.

COEXISTENCE OF OVARIAN TUMORS WITH OTHER TUMORS

As emphasized in previous papers of this series, mice with tumors in one tissue exhibit tumors in other organs with a frequency greater than would correspond to the average incidence of tumors. It may be recalled that of the 8 cases of ovarian tumors recorded in the literature, in 4 there were tumors elsewhere in the body.

Among our 40 mice with benign ovarian tumors, 22 had tumors elsewhere, and frequently these were multiple tumors. Of our 4 mice with primary malignant ovarian tumors, all had tumors elsewhere; two mice having each two carcinomas of the mammary gland, one having a single carcinoma of the mammary gland, and one a papillary adenoma of the lung which had invaded a blood-vessel, being therefore presumably malignant. The additional tumors in the 26 mice with both ovarian and other tumors, were located as follows:

Sixteen had one or more primary carcinomas of the mammary gland.

Four had papillary adenoma of the lung.

Two had papillary adenoma of the lung and carcinoma of the mammary gland.

One had adenoma of the mammary gland.

One had a carcinoma and a sarcoma of the mammary gland.

One had a subcutaneous sarcoma.

One had a subcutaneous sarcoma and an osteosarcoma.

SUMMARY

Among 22,000 mice of the Slye stock dying natural deaths at all ages were 44 mice with spontaneous primary ovarian tumors not including simple ovarian cysts. Of these, 38 had simple benign solid papillary adenomas, only occasionally with slight cyst formation. One showed a typical papillary cystoma. One had a typical solid teratoma containing a great diversity of tissue elements.[6] Of the 38 cases of solid papillary adenomas, 19, or 50 per cent, were bilateral, so that there were 57 tumors of this class. There were 4 unquestionably primary malignant tumors of the ovary, all showing the ''mesothelioma'' type of growth characteristic of malignant tumors derived from the sex glands; one of these produced perirenal metastases. There was one other tumor of the same type primary in either the ovary or the adrenal. Two round-cell sarcomas were found that arose either from the ovary or some other organ, while two other sarcomas had produced secondary growths in the ovary. Of the 44 mice with primary ovarian tumors, 26 had tumors in other parts of the body.

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[6] A second case of teratoma has been observed since this analysis was made.

In the literature were found reports of 8 other cases of tumors arising in the ovaries of mice, which exhibited quite the same characteristics as the tumors described in this paper.

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RHABDOMYOMA OF THE OVARY

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Tumors of striated muscle are sufficiently rare to make the observation of a new and characteristic case worthy of record. The rhabdomyoma which is the subject of the present discussion is of special interest because of the peculiar forms assumed by the myogenic cells and the wide variations of structure in the tumor.

At the present time a detailed discussion of the literature of this neoplasm is unnecessary; but the facts which are of more direct bearing on the present case may be mentioned. Benenati's list of 65 cases was published in 1903; and the cases reported since then do not show any material change in the relative frequency of this tumor in the various parts of the body. They may be divided according to the regions in which they occur. Rhabdomyoma is found most often in the genitourinary tract. There are 39 cases occurring in this region: kidney, 13; testis, 9; uterus, 6; pelvis of the kidney, 3; vagina, 3; bladder, 3; ovary or uterus, 1; ovary, 2. The tumor of the ovary described by Virchow in 1850 was a papillary cystic rhabdomyosarcoma, some of the papillae being formed of striated muscle. A second rhabdomyoma of the ovary was reported by Vignard. It was similar to the tumor about to be described, the greater part being striated muscular tissue with cystic degeneration at one extremity.

Wolfensberger noted the frequency of this tumor in the neck and adjoining regions, which stand second to the genitourinary tract with 9 cases. These localities are: orbit, 2; temporal bone, nose, tongue, parotid, mandible, esophagus, and mediastinum. Four examples are found posterior to the pelvis. They were in the lumbar region, hip, ischial tuberosity, and anus.

Homologous rhabdomyomas are found with greatest frequency in the heart, where 8 cases are recorded. Recently Wolbach has added an instructive case to the list. The remaining 5 examples were found in the following regions: pectoralis major, breast, shoulder, elbow, and thigh.

Muller has recently published a case in which rhabdomyosarcoma followed successive fractures of the femur. In his case the tumor probably arose from previously normal adult voluntary striated muscle cells. Such tumors, while probably not infrequent, are rarely reported, and belong to a group entirely different from the ordinary heterologous or teratomatous rhabdomyoma.

Clinically, rhabdomyomas are tumors of moderately rapid growth. Most of the tumors are well encapsulated and form no metastases; however, there are others with infiltration of surrounding tissues and recurrence after removal (Billroth, 2 cases; Buhl, Kaschewarowa) and some which formed metastases (Wolfensberger, Eberth, Benenati). Burgess has reported a case in which there were multiple metastases throughout the body.

Cohnheim's theory of embryonal rests has been accepted by many authors as accounting for the genesis of this tumor, especially of the heterologous rhabdomyomas. Ribbert has suggested as the origin of his renal tumor the development of striated muscle from the smooth muscle present in the pelvis of the kidney. His arguments do not seem to be convincing.

Benenati gives five possible derivations for rhabdomyoma testis. It may arise from smooth or striated muscle derived from the cremasteric muscle or from the gubernaculum; he finally concludes that the new growth arises from an embryonal rest. In 1904, Ribbert came to the conclusion that rhabdomyoma testis is a one-sided development of a teratoma. In the present communication I hope to extend this interpretation to rhabdomyoma of the ovary.

The genesis of teratoma has been variously explained. Verneuil in 1855 agreed with other writers in finding the origin of this tumor in a twin inclusion. Theories which found the source of tridermal neoplasms in polar bodies and isolated blas-

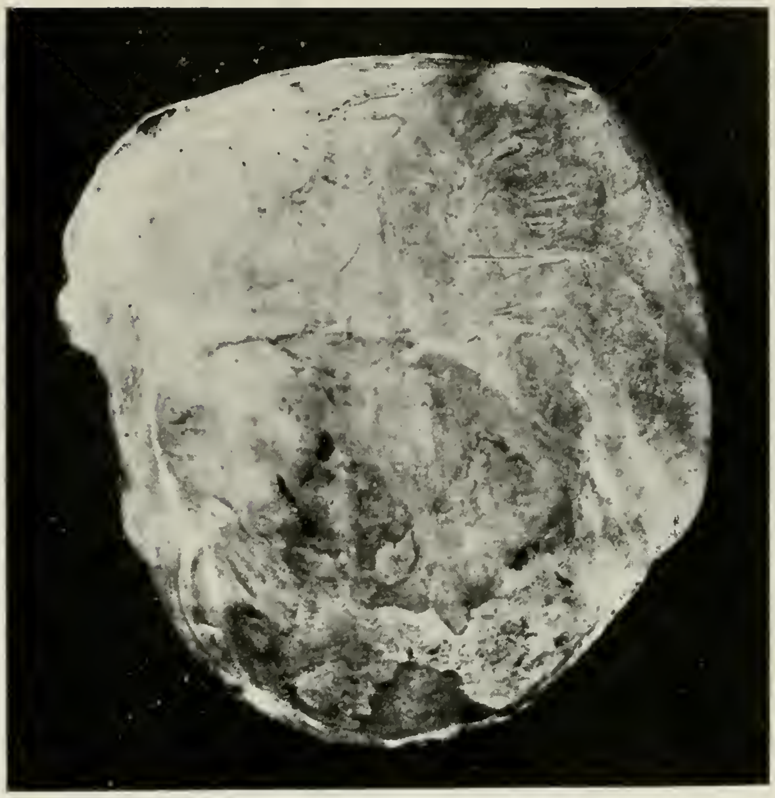

FIG. 1. GROSS SECTION OF TUMOR

Note capsule and thin strands of connective tissue surrounding the individual spheroidal masses which make up the tumor.

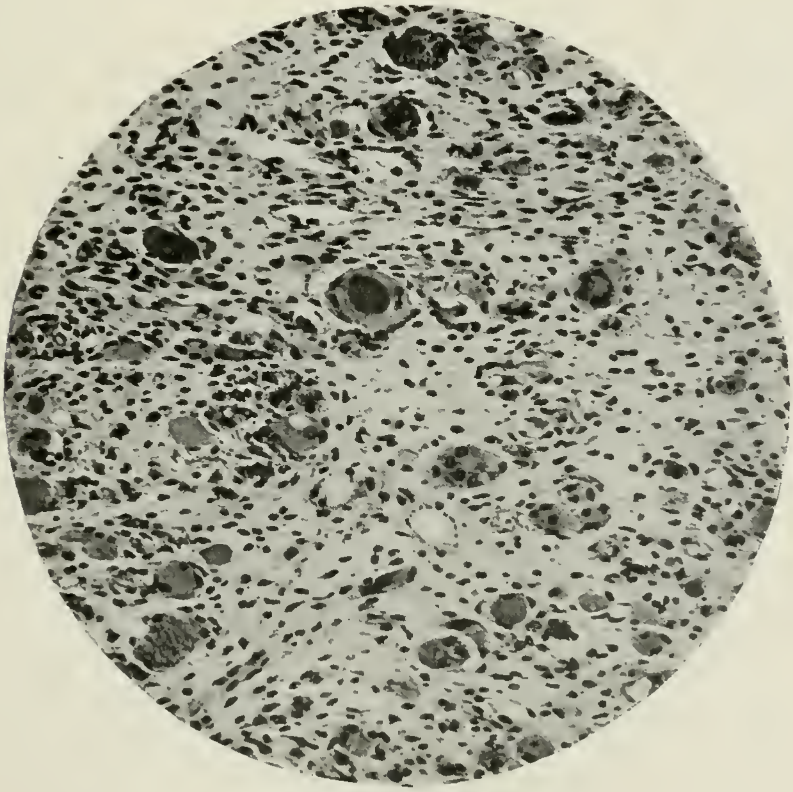

FIG. 2. LOW POWER FIELD, INCLUDING THE CELL SHOWN IN GREATER DETAIL IN FIGURE 5

The giant cells are surrounded by myxomatous tissue. In large portions of the neoplasm the latter element is found exclusive of any other.

tomeres have given way to one which is based on the parthenogenetic development of the sex-cell. At the present time the last mentioned theory has the greatest number of adherents.

The results of experiments performed by Stockard have led me to believe that the twin inclusion theory will best explain the origin of this neoplasm. In the following I shall endeavor to prove that this theory is applicable not only to rhabdomyoma of the ovary, but to most rhabdomyomas in common with many simple tumors occurring inferiorly, either in front or behind the pelvic zones, in the genitourinary tract, or superiorly, in the neck region.

Present case. October, 1918, Edith M., one and a half years of age was found to have a mass in the abdomen reaching half way to the umbilicus. In shape and size it seemed to resemble a kidney with the long diameter horizontal. It was freely movable. There were no subjective symptoms. At the time of the operation nine months later, the mass appeared to fill the whole abdomen up to the umbilicus. Early in February, 1919, the child begun to vomit to such an extent as to cause the mother to give consent to operation. At that time a yellowish discoloration appeared on the skin about the umbilicus.

Operation, performed February 9, 1919, disclosed a well encapsulated tumor arising apparently from the region of the left ovary, filling the pelvis, with a narrower upper portion lying under the inferior surface of the liver. The capsule was attached to the anterior abdominal wall posterior to the umbilicus. The entire new growth was removed. In the process of removing the tumor the capsule was broken so that some of the myxomatous tissue fell into the abdominal cavity. The child died of abdominal recurrence, May 31, 1919.

Gross anatomy. The tumor is covered by a thin, movable capsule and consists of two parts, a lower, hard, spherical mass, 11 cm. in diameter, and an upper myxomatous portion, 4 cm. in diameter. It weighs 26 ounces. The surface is smooth and presents various rounded protuberances. On section of the larger part, the capsule is seen to run inward from the surface in thin strands throughout the tumor, giving the impression that it was the covering of an ever increasing growth. The tumor is made up of rounded masses, 3 cm. to a few millimeters in diameter, suggesting that it grew by the appearance of new parts, as well as by the increase in size of the older portions. On section the myxomatous division was found to contain a cavity.

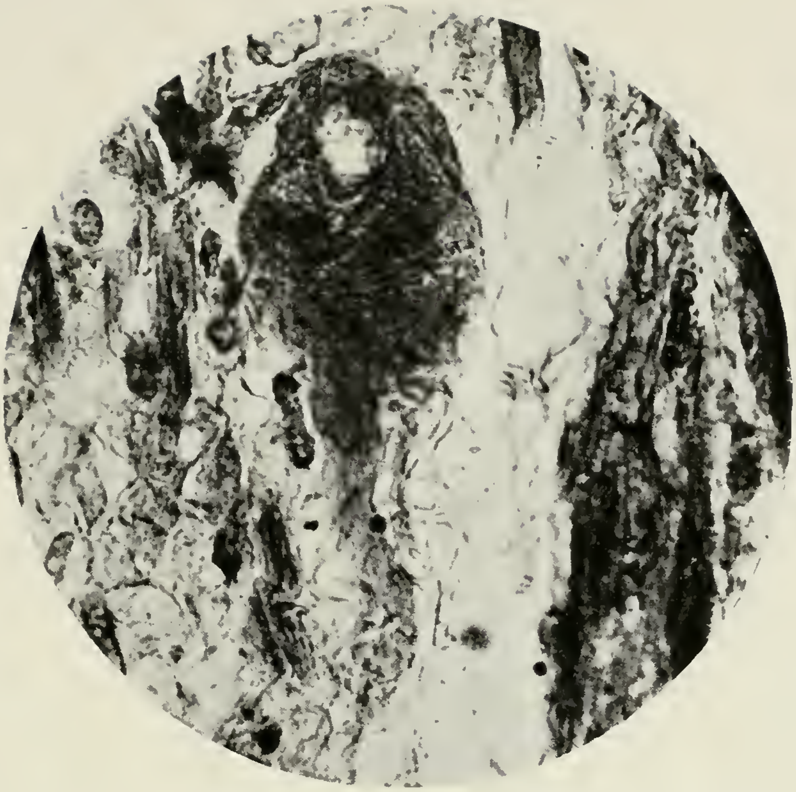

FIG. 3. GIANT CELL WITH EXTENSION OF FIBRILLATED PROCESS IN MUSCLE FIBER FORMATION

Adjoining are muscle fibers showing to some degree longitudinal and cross striation.

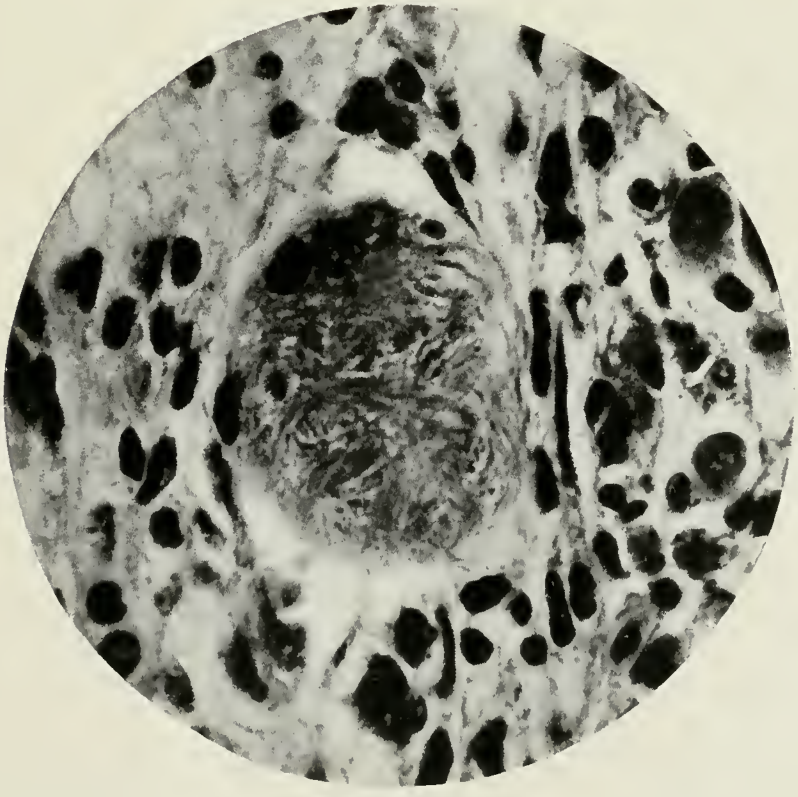

FIG. 4. GIANT CELL WITH WELL-FORMED FIBRILS ARRANGED WITHOUT DEFINITE ORDER

There are four nuclei situated at one end of the cell

Structure. Several main histological features deserve description. Perhaps the most striking are the *giant cells.* These cells have an acidophile cytoplasm in which usually there are concentric striae, most prominent at the periphery, while the perinuclear cytoplasm is granular. The nucleus or nuclei are round or broadly oval, sometimes containing 1 to 3 nucleoli. In other cells one or more centrosomes, as shown in figure 6, give evidence of active mitosis.

Muscle fibers. There are areas in the tumor composed of long cells with the characteristics of muscle fibers. The nuclei are oval and usually situated in the median axis. The peripheral cytoplasm is fibrillated, but only rarely can any cross striations be made out. Some of the fibers branch.

Myxomatous tissue. In several parts star-shaped cells with nuclei the size of those seen in the muscle fibers, and fibrils extending radially from them, can be seen separating the muscle fibers, showing that they developed alongside each other. In other places, they are the only element seen in large areas, giving the tumor a distinctly myxomatous appearance.

Histogenesis. The normal embryology of muscle may be taken as a guide to the anaplastic process. Striated fibers arise from myoblasts which elongate and by repeated mitotic division of their nuclei form a syncytium. The nuclei are surrounded by granular cytoplasm in which fibrils differentiate peripherally. The myofibrils became striated. The fibers increase in size and the nuclei migrate to the periphery. Heart muscle undergoes a similar development; but the nuclei remain centrally situated, sarcolemma never develops, and the individual syncytia are in connection with each other. Assuming that the tumor arose in a cell or group of similar cells we might expect to be able to follow the development till we arrive at the picture given in the histological description. Examining the three elements of the tumor it is possible to discern such an unfolding.

Giant cells. The giant cells as a group show a fundamental error in development; they have failed to elongate.

1. There are giant cells which show the extension of a process in an abortive development of muscle fiber (fig. 3).

2. There are giant cells which never elongated, while in other respects, they went through a more or less normal and complete development (fig. 5).

3. There are others which show increasing anaplasia until we come to some in which the fibrils lack definite order (fig. 4).

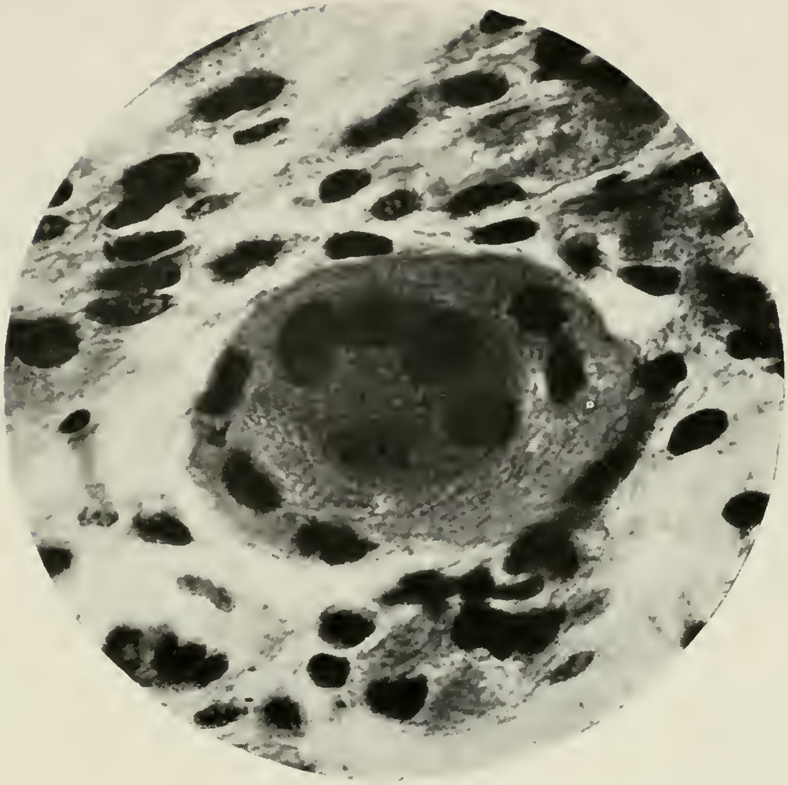

FIG. 5. THIS CELL DID NOT ELONGATE, BUT IN OTHER RESPECTS IT WENT THROUGH A COMPLETE DIFFERENTIATION

The fibrils and cross striae are shown as concentric and radial striae

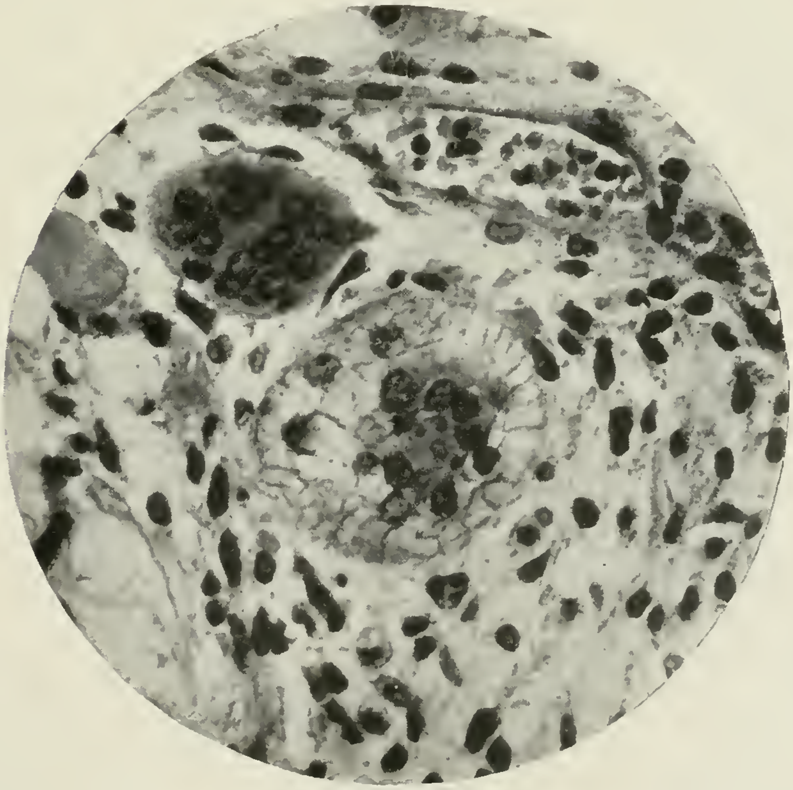

FIG. 6. "SPIDER CELL" IN WHICH THE CYTOPLASM BETWEEN THE PERIPHERY AND THE NUCLEI HAS DEGENERATED AND BEEN ABSORBED

The perinuclear cytoplasm contains five centrosomes. This cell occurs only in cardiac rhabdomyoma.

I am indebted to Mr. William Dunn for the photographs.

4. Lastly, there is a group of cells, some of which resemble the "spider-cells," first described by Cesaris-Demel in a remarkable tumor, a rhabdomyoma of the heart (fig. 6). Others lie in spaces which Wolbach showed to be intracellular. Seiffert concluded that these spaces were formed within the peripheral differentiated portion of the cell by the degeneration and disappearance of the greater part of the remainder of the cytoplasm. The histogenesis of the "spider-cells" is similarly explained.

The fact that this type of cell has been found exclusively in rhabdomyoma of the heart, and the fact that I have found branching in some of the fibers, have led me to conclude that the present tumor arose from heart muscle. Katsurada, among others, reported heart muscle in dermoid cysts. It should be added that while sarcolemma is occasionally developed in rhabdomyomas, it is never found in those of the heart. Also, here as in heart muscle, the perinuclear cytoplasm remains undifferentiated. Some writers have described branching in other rhabdomyomas. This may indicate that in an anaplastic process such forms may be evolved, or that these other neoplasms had an origin similar to the one now being described.

The second element of the tumor need not long detain us. The muscle fibers are descendants of cells which developed more normally than those heretofore mentioned.

There are many cells which show intermediate stages of development between the muscle fibers and myxoma cells. The genesis of the myxoma is explained as follows: it occurred in the most rapidly growing portion of the tumor, and, as is well known, parallel with an increasing rapidity in the multiplication of cells there is a corresponding loss in differentiation.

In this laboratory there are three other rhabdomyomas with a mucous element very similar to the one described. The literature affords other cases in which myxoma complicated an otherwise pure rhabdomyoma (Billroth, Kaschewarowa, Targett). The possibility thus arises that with the preponderance of myxomatous tissue a rhabdomyoma may be misinterpreted as a pure myxoma.

In some parts of the tumor surrounded by myxomatous tissue, the nuclei disappear, leaving areas of pure fibrils. These areas of fibrils raise the question of the possibility of the multiplication of fibrils without the corresponding multiplication of the myxoma cells. An analogous condition seems to obtain in neurocytoma, in which a similar picture of excessive fibrils may appear. It does not seem possible for the multiplication of fibrils to take place without the corresponding division of nuclei, but the overgrowth of fibrils of the individual cells is quite possible. A similar overgrowth of cytoplasmic derivatives is seen in the formation of epithelial horn.

Origin of the tumor. 1. Having shown that all the elements may be derived from myogenic cells, the next question to be considered is the origin of the group of cells from which the tumor arose. In considering this heterologous neoplasm we may leave a metaplastic origin out of the question. This leaves two possibilities: the tumor arose (a) from an embryonal rest; or (b) as a one-sided teratoma.

We may safely eliminate the former possibility. In general, nothing is known about embryonal muscle rests in the ovary. In particular, the organ is not situated near striped muscle so as to make such a rest possible. This conclusion becomes more evident since we are dealing with cardiac muscle.

It is an accepted fact that one element of a teratoma may outgrow the others (Pick and Walthard) and that the neoplasm may be represented by a single tissue. In the case of rhabdomyoma this conclusion gains added strength when we remember that striated muscle sometimes identified as cardiac, is often found in teratomas. It is interesting to note that Cornil thought the rhabdomyoma described by Vignard, the only case in the literature similar to the present one, was a fetal inclusion.

Additional support is found in the occurrence of similar tumors in an analogous organ. Rhabdomyoma testis is admittedly of teratomatous origin.

2. Since the teratomatous origin is the only one which will explain the rhabdomyoma it is necessary to determine in what manner the teratoma arose, and again there are two possibilities; it arose either from a sex cell or from a twin inclusion.

In the ovary, where there are totipotent sex cells, it may seem unnecessary to seek further for the origin of the teratoma. The commonly accepted explanation is the parthenogenetic development of this cell. This view point is open to criticism because mammalian ova, with the exception of one series reported by L. Loeb, have never been observed to develop parthenogenetically and then continue a separate existence. Loeb, himself, claims little vitality for these ova, while Bandler and Wendeler quote other investigators to the effect that ova which undergo such a development invariably end in complete degeneration.

On the other hand, the twin inclusion theory has a basis in experiments recently performed by Stockard. By slowing the rate of development of fish embryos at the time when the primary bud should arise, two or more buds may develop simultaneously. The degrees of doubleness of the resulting individual is determined by the distance between the two buds on the blastodisc. At 180°, that is diametrically opposite each other, two complete individuals are formed.

When one bud gets the start of the other by any advantage it obtains a supremacy which allows it to develop into a perfect individual. The component arising from the inferior bud is suppressed and interfered with so that it develops abnormally. The twins are attached anteriorly in the head and neck region, or posteriorly, the great majority of them in the latter situation. There are all degrees of this inhibition of the second twin until it is reduced to a mere fragment of included tissue. The point made by Stockard is that human monsters have been born which show exactly the same relation to each other, as above described. Monsters attached superiorly on the head, neck, or upper thorax, or inferiorly near the sacrum, or anteriorly in the ventral region, can be traced in an unbroken series until the inhibited twin is represented by a teratoma or dermoid.

On the basis of these experiments it is possible to explain the presence and frequency of rhabdomyomas in the regions given in the first part of the paper, that is, 9 superiorly in the neck region, 43 inferiorly in the pelvic region.

No facts have been brought forth to disprove the twin inclusion theory. However, there are two questions which need further discussion. The first is the great frequency of teratomas in the sex glands; and the second, the occurrence of chorioma in males and in females at such a time as to shut out the possibility of impregnation. These phenomena can be explained by the twin inclusion theory.

Bonnet remarked the fact, in defending the theory of the origin of teratoma from an independent blastomere, that if a blastomere should separate from the remainder of the blastula, the organ to which it would be most likely to attach is the mesonephros.

For a long time the mesonephros is the largest organ in the abdominal cavity. It is the most vascular. Later the mesonephros survives as functional and vestigial parts of the genital system; in the male, the efferent ducts, the paradidymis, epididymis and vas; in the female the paroöphoron, parovarium, and Gartner's ducts.

If the twin were attached to the mesonephros, we should expect to find it adherent in the testis to the mesonephric tubules which join those of the sex gland. This is actually the case, for Ewing in his paper on teratoma testis points out that almost invariably the neoplasm arises in the neighborhood of the rete. We can explain those which occur in the scrotum external to the tunica albuginea by the accidental attachment to other portions of the mesonephros. Bandler has made an attempt to explain teratoma as a development of the paroöphoron. We now see that while it does arise from this region it is not a development from this organ, but is rather attached to it.

The position of teratoma is explained by the organogenesis of the sex glands in which the mesonephros plays such a prominent part. This accounts for most teratomas attached anterior to the pelvis. It explains the 39 rhabdomyomas found in the genitourinary system. The other four, being situated posteriorly, were not able to become adherent to the mesonephros. From these facts arises the thought that many simple tumors of the ovary may be of teratomatous origin. Ewing has come to the conclusion that practically all common tumors of the testis are of similar origin.

In taking up the question of chorioma, we may again turn to an observed fact. Up to the present time nobody has been able to cause the spermatozoa of any species to develop into an embryo. How then can we explain chorioma testis except as an early inhibited twin with the predominance of this single chorionic tissue?

Boestrom reported a case of chorioma with multiple metastases throughout the body in which the testicles were normal. Djewitski recorded a case of chorioma of the bladder in a virgin, seventy-five years old. Surely rather late for the parthenogenetic development of an ovum, but not against the growth of a long repressed twin. The facts of extragenital chorioma can be explained best as a twin inclusion. The relations between the two components is such that with the growth of one there is an inhibition of the other. The rate of development of the host after birth is much retarded, and the repressed component is given the required opportunity to grow. This explains why the majority of teratomas begin their growth in early life rather than during the longer adult period. Senile atrophy of the host gives the parasite a last chance for development.

Causal genesis. The frequency of teratoma testis after trauma is well known. This may not only directly precipitate the rapid growth, but indirectly, by lowering the vitality of the organ, give the long repressed twin inclusion the temporary advantage. The real causal genesis lies in the potential energy for growth constantly waiting a chance for expression. Further, that the growth should be autonomous is the only possibility in tissues which have been so disorganized. This may be capable of experimental verification.

To sum up the points of the theory of the twin inclusion: It is based on observed facts and therefore can give a true casual genesis. It explains the position and frequency of teratomas, in the sex glands as well as elsewhere in the body, obviating the necessity for multiple explanations for teratomas. The theory holds for all one-sided teratomas.

The possibility of new evidence in favor of any other explanation for the frequency of teratomas in the sex glands will not disprove the twin inclusion theory, but will be complementary to it. Likewise the theory holds independently of the method of the formation of the twin.

According to the theory of twin inclusion, the complete sequence of events is as follows: an ovum is fertilized; through some unfavorable condition the rate of development is slowed; two primary embryonic buds are formed on the blastodisc, one having the advantage over the other. The favored bud develops into a perfect individual, during which process the smaller bud is inhibited and disorganized. The latter, being united to the more vigorous component at its ventral region, becomes adherent to the mesonephros; hence, later it forms a part of the ovary. With the birth of the child and its consequent diminished rate of development, the inhibited twin is given a new opportunity to grow. Through its previous disorganization it is not capable of orderly development. The heart muscle grows more rapidly and succeeds in crowding out the other elements. The growth gains in momentum, and myxoma cells are formed which have no resemblance to the earlier muscle cells.

SUMMARY

The case described is one of rhabdomyoma of the ovary in an infant.

Three elements arose from one tissue by (a) histogenesis more or less normal; (b) anaplastic development; (c) degenerative changes. Although only one tissue, cardiac muscle, is present, the tumor is of teratomatous origin. Many simple tumors of the ovary are probably of similar origin. Many tumors of the head, neck, thorax, and genitourinary and posterior pelvic regions are of teratomatous origin. This group includes the great majority of heterologous and some of the homologous neoplasms.

Teratoma is a twin inclusion. A group of cells is found in this tumor which appears only in rhabdomyoma of the heart.

Therefore, the present tumor is a rhabdomyoma of the heart of a twin inclusion. Fibrils may be produced in such overabundance as to lose connection with their nuclei and seem to be multiplying independently. Myxoma may be secondary to rhabdomyoma.

I wish to thank Dr. Stockard and Dr. Ewing for help in the preparation of this paper.

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MALIGNANCY OF THE CROWN-GALL AND ITS ANALOGY TO ANIMAL CANCER[1]

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METHODS EMPLOYED IN THE INVESTIGATIONS

In 1907, Dr. E. F. Smith showed that the disease of plants called crown-gall—tumor-like in nature—could be reproduced artificially by an inoculation of a normal plant with a pure culture of a bacillus which he called Bacterium tumefaciens. Smith maintains that this or a similar disease can be produced only by this particular organism, that all other tumor-like formations observed in plants due to other parasites, like Plasmodiophora brassicae, are entirely different pathological entities, and that only crown-gall is a true plant tumor. In his estimation, this tumor is practically identical, biologically, with animal and human cancer. Since crown-gall is caused by the action of the bacterium described by him, he concludes in one of his recent articles that "to a biologist the conclusion is almost irresistible that human cancer must be due to a parasite and that one parasite may well be the cause of the most diverse forms, as we have seen to be the case in plants." This deduction is so sweeping in character, so far-reaching in its generalization, and the results of Dr. Smith's work have become so widely known and quoted by the medical profession that it has seemed to the writers to be desirable to renew his investigations, to analyze the material from the viewpoint of animal pathology, and to attempt to determine the true analogy between the crown-gall and animal cancer.

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[1] Presented before the American Association for Cancer Research, June 14, 1919.

The writers have inoculated with the pure culture of Bacterium tumefaciens the following plants: Growing tips, stems, petioles, and blades of Rosa (Rose), Ricinus (castor oil), Pelargonium (house geranium), Lycopersicum (tomato), Nicotianum (tobacco), Helianthus (sun-flower), Bryophyllum calycinum and Ficus elastica (India-rubber tree). Galls formed were fixed at various stages of development in Carnoy, Flemming strong, Hermann, Merkle, and Bouin's solutions. The first fixation was found best suited for the more woody galls. After embedding in paraffin, the sections were cut from 5 to 30 micra in thickness, and were stained with Flemming's triple stain, methyl green counterstained with fuchsin, or Delafield's hematoxylin counterstained with eosin. The most satisfactory preparations were obtained by fixing with Carnoy solution and staining with gentian violet and safranin. In these preparations the cell walls stain a

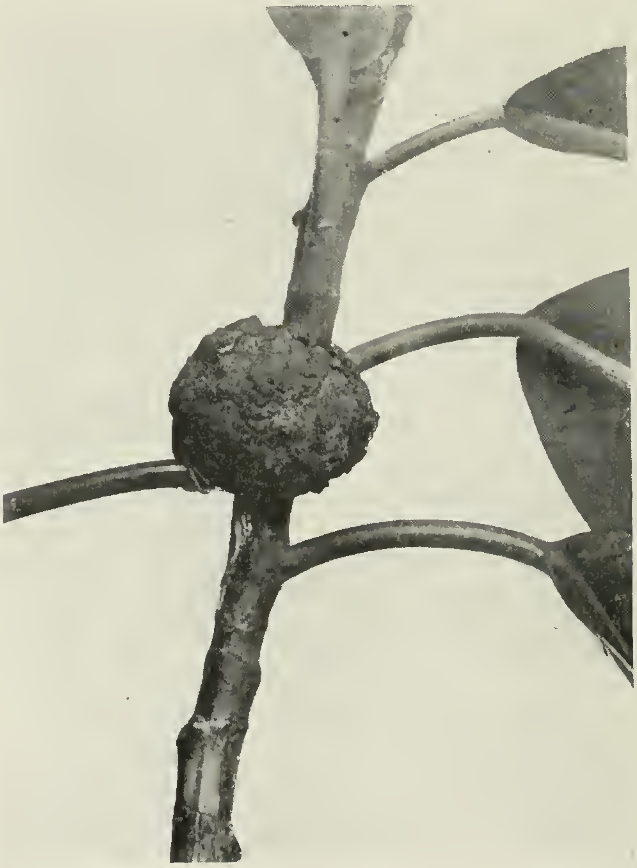

Fig. 1. Crown-Gall on a Stem of a Rubber Plant

beautiful deep violet, while the cytoplasm and chromatin material stain a much fainter purple. The nucleolus takes a bright ruby red color.

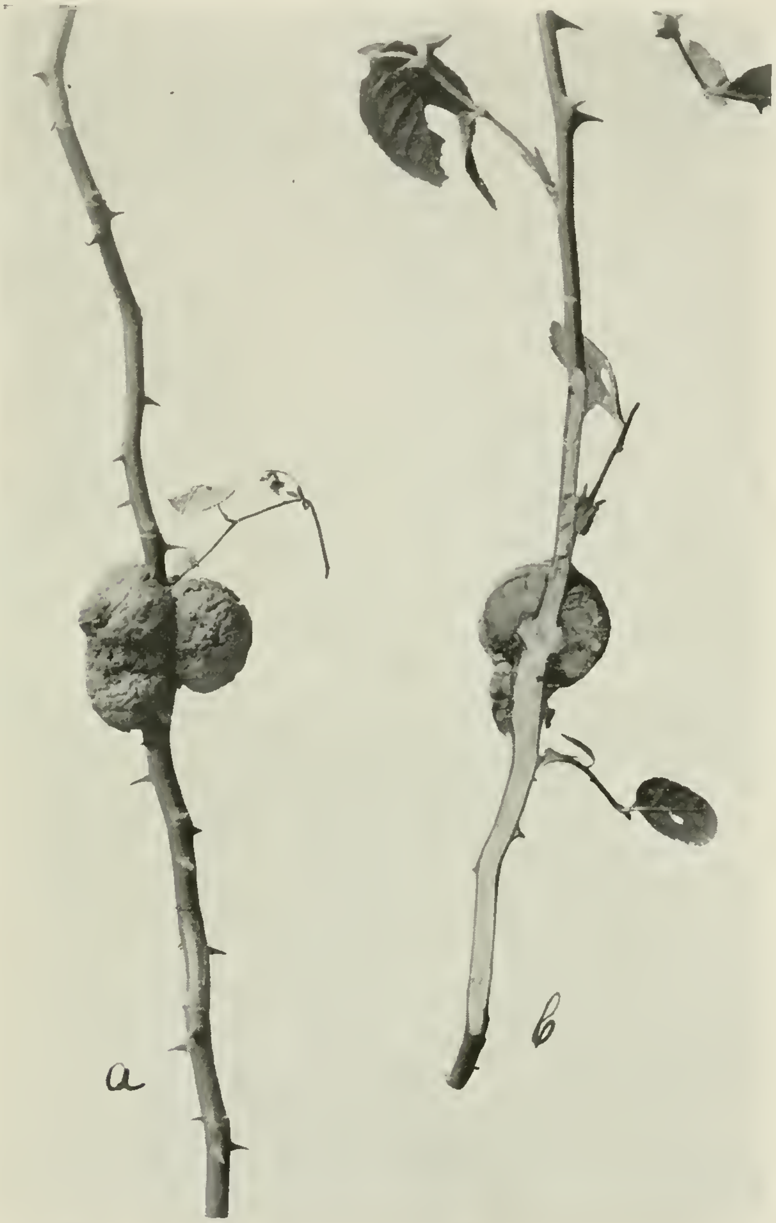

Fig. 2. *a*, Crown-Gall on a Stem of a Rose; Wart-like Benign Gall. *b*, Longitudinal Section of the Same Stem

The gross study of the material shows that in nearly every inoculated plant there developed a distinct crown-gall within one or two months after inoculation. Usually these galls grow slowly and are not very large in comparison with the size

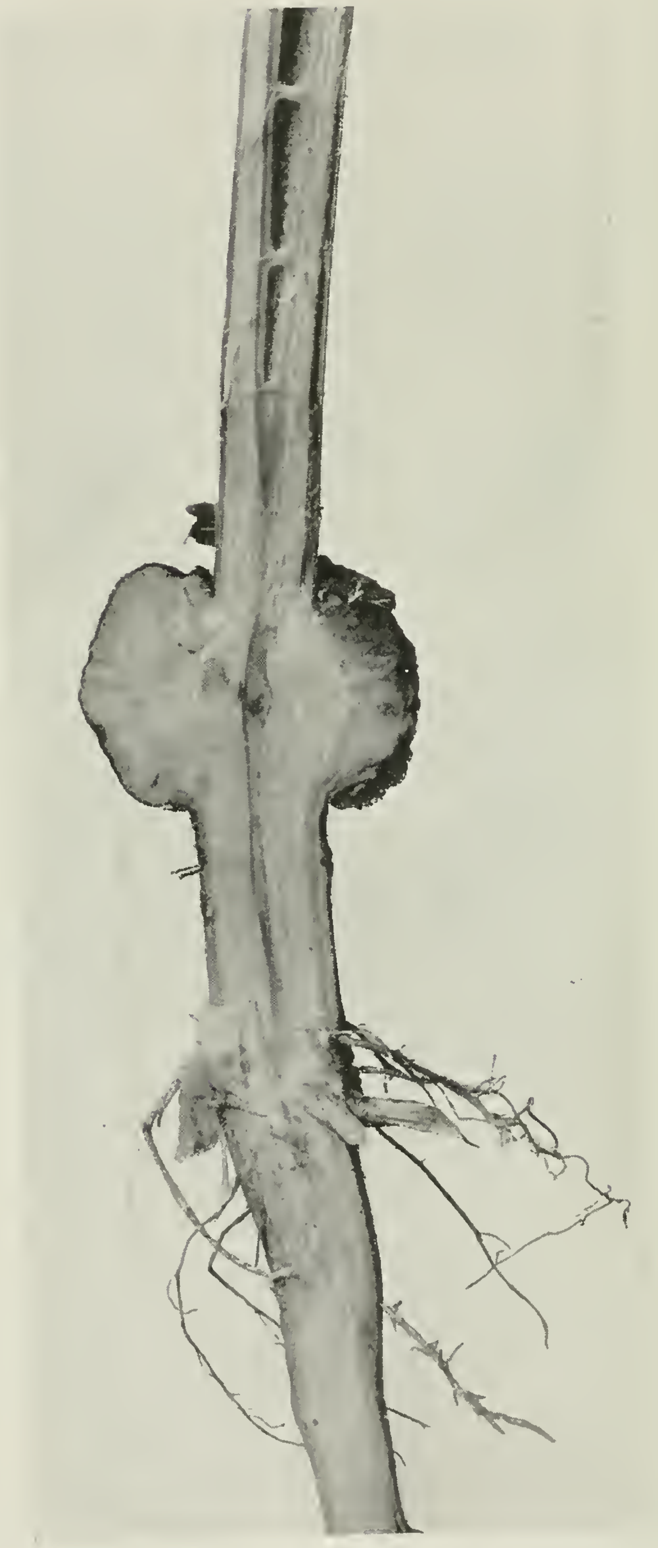

FIG. 3. LONGITUDINAL SECTION OF A STEM OF RICINUS PLANT
The gall is benign, the normal stem tissue is only displaced

of the plant itself. Figure 1 shows a gall which grew for over six months on a rubber plant; the plant itself is 6 feet high. Most frequently the gall grows out of the surface of the stem and does

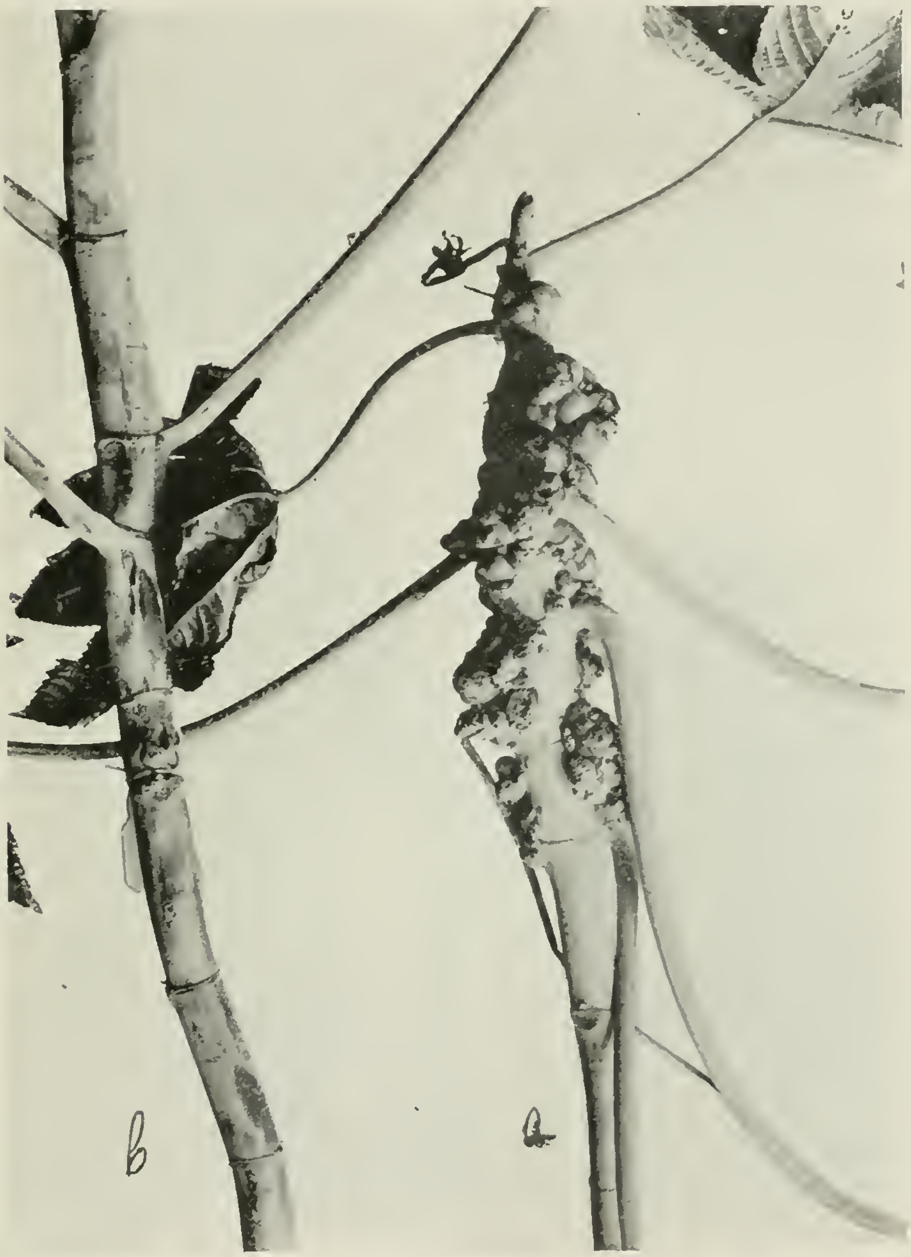

FIG. 4. *a*, RICINUS PLANT INOCULATED AT THE APICAL PORTION OF THE STEM; SHOWS DWARFING OF THE PLANT. *b*, CONTROL PLANT

not seem to affect the rest of the plant (fig. 2). In other instances the growth displaces and distorts a part of the cortical area of the stem (fig. 3). Only in a small percentage of the inoculated plants does the crown-gall affect the tissues of the plant more deeply. Figure 4 shows a photograph of the growing regions of two plants, one inoculated with the microorganism, and the other a normal control plant. While the latter grew up above the tip, the inoculated plant ceased to grow and became dwarfed, and the leaves drooped. At the same time, even in the inoculated plant, the lower part of the stem as well as the root apparently remained normal. Figure 5 presents an instance where the part of the plant above the point of inoculation, and even to a certain

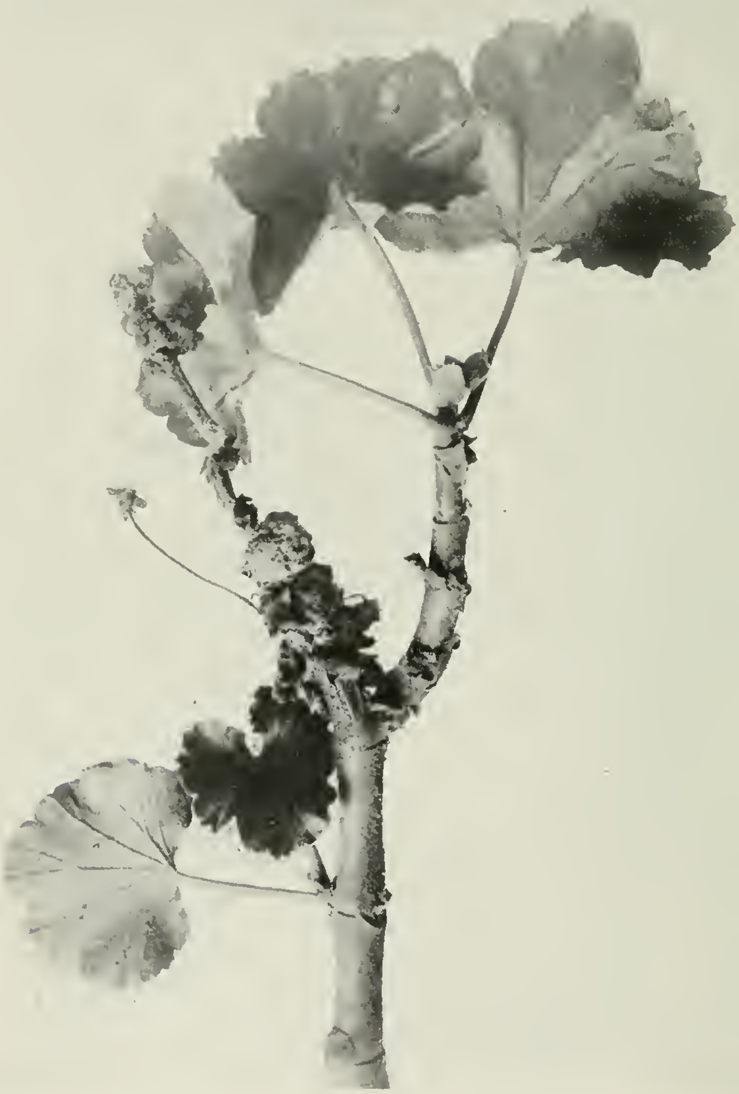

Fig. 5. Pelargonium
Shows necrosis of the inoculated part of the plant

degree below the point of inoculation, became necrotic. Cross (fig. 6) or longitudinal (fig. 7) sections of such plants show that these crown galls grow invasively, destroy and replace normal plant tissue, and occasionally develop further along the length of the stem in a manner which produces the appearance of a secondary metastatic tumor above or below the primary growth (fig. 8).

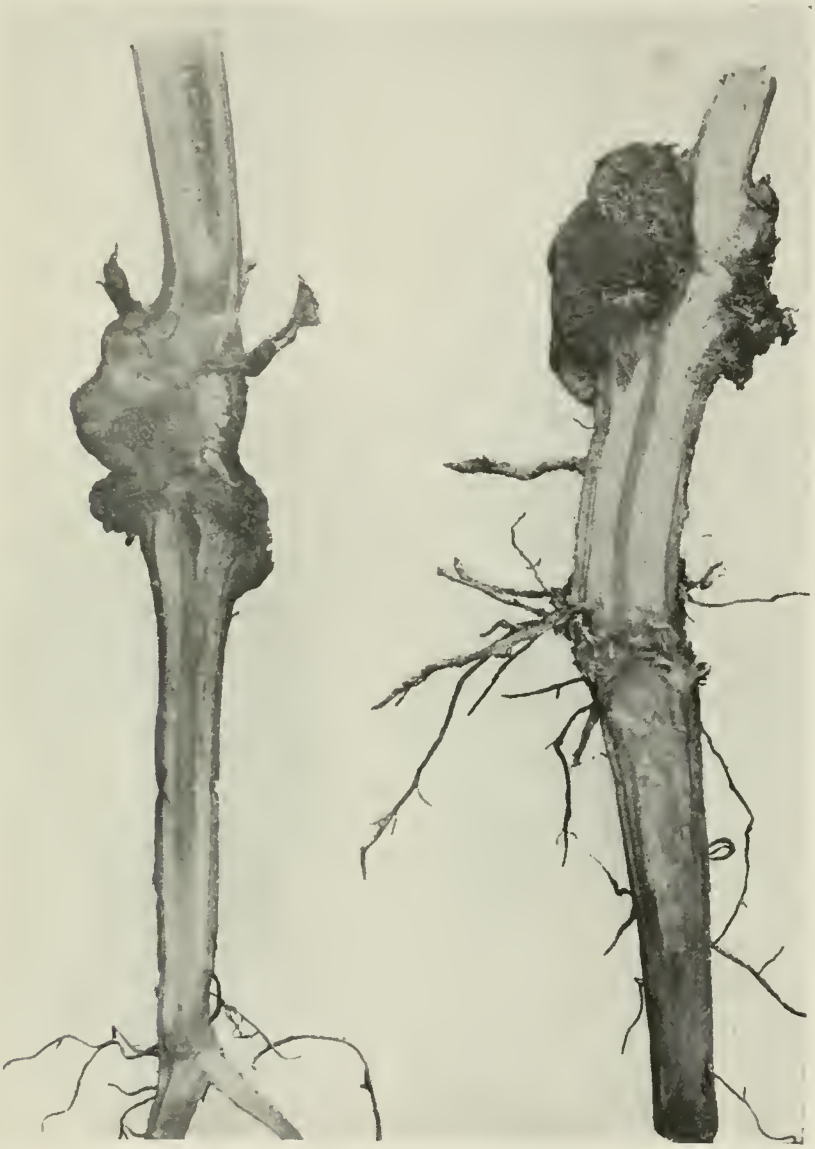

Fig. 6. Longitudinal Sections of Two Stems of Ricinus Plants
Show invasive growth of the gall and destruction of the normal stem tissue

In accordance with the findings of Smith, the writers have also observed in many instances the formation of small leafy shoots at the periphery of a crown-gall (figs. 9 and 10).

The microscopical analysis of the material shows that the crown-gall consists of a number of uniformly small, young, undifferentiated cells which contain a greater amount of cytoplasm than the normal parenchyma cells within which they develop (fig. 11). The nuclei frequently show mitosis.

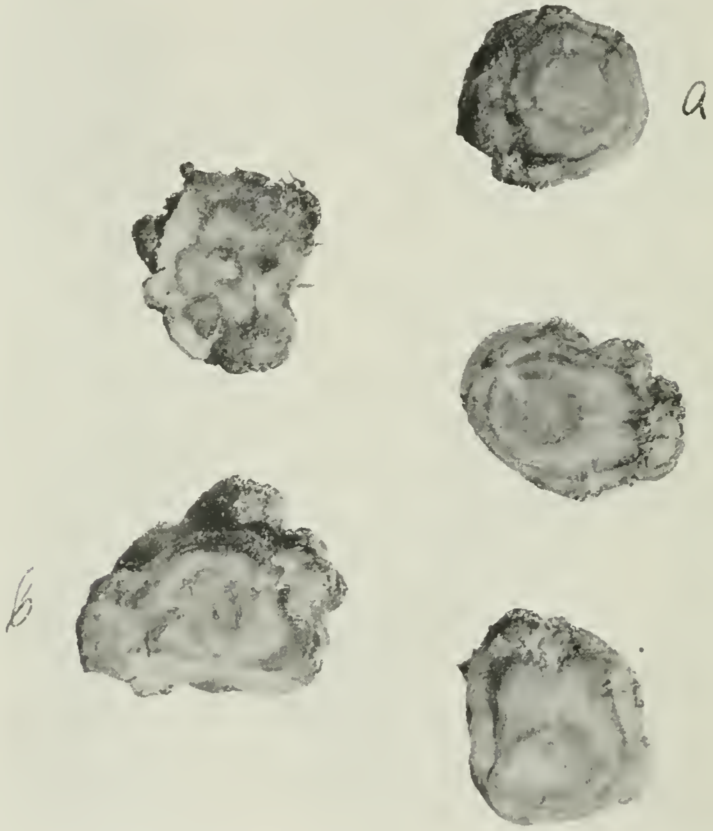

Fig. 7. Cross Section Showing the Gradually Increasing Invasion of the Normal Stem

The invasion is least at *a* and greatest at *b*

The small slowly growing crown-galls which frequently develop when the inoculation is done at the bases of leaves (fig. 12) present a very striking picture when examined microscopically. In the center of the growth there is an area of characteristic crown-gall cells, and surrounding this area the crown-gall cells appear to be much larger in size and present all the characteristics of normal parenchyma cells (fig. 13). Apparently in these crown-galls the peripheral gall cells became differentiated into adult

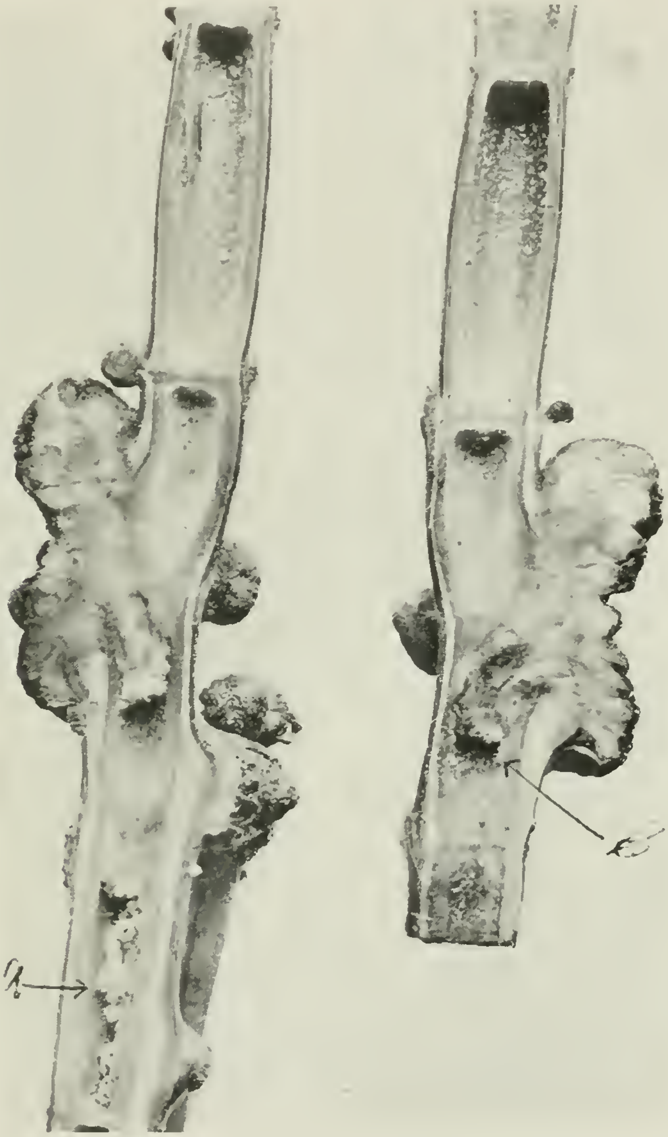

FIG. 8. LONGITUDINAL SECTIONS SHOWING AT *a* AND *b* CONDITIONS SOMEWHAT SIMILAR TO METASTASIS FORMATION

normal tissue cells. Frequently there were observed new irregular conducting systems formed within the crown-gall tissue (fig. 14), also apparently as a further differentiation of the crown-gall cells.

Microscopical study of a large number of the leafy sheets which developed at the periphery of a crown-gall showed that the starting point and the base of each leafy shoot is ordinary crown-gall tissue with the identical gall cells (fig. 15), indicating that the crown-gall cells themselves gave rise to the formation of the leafy shoot.

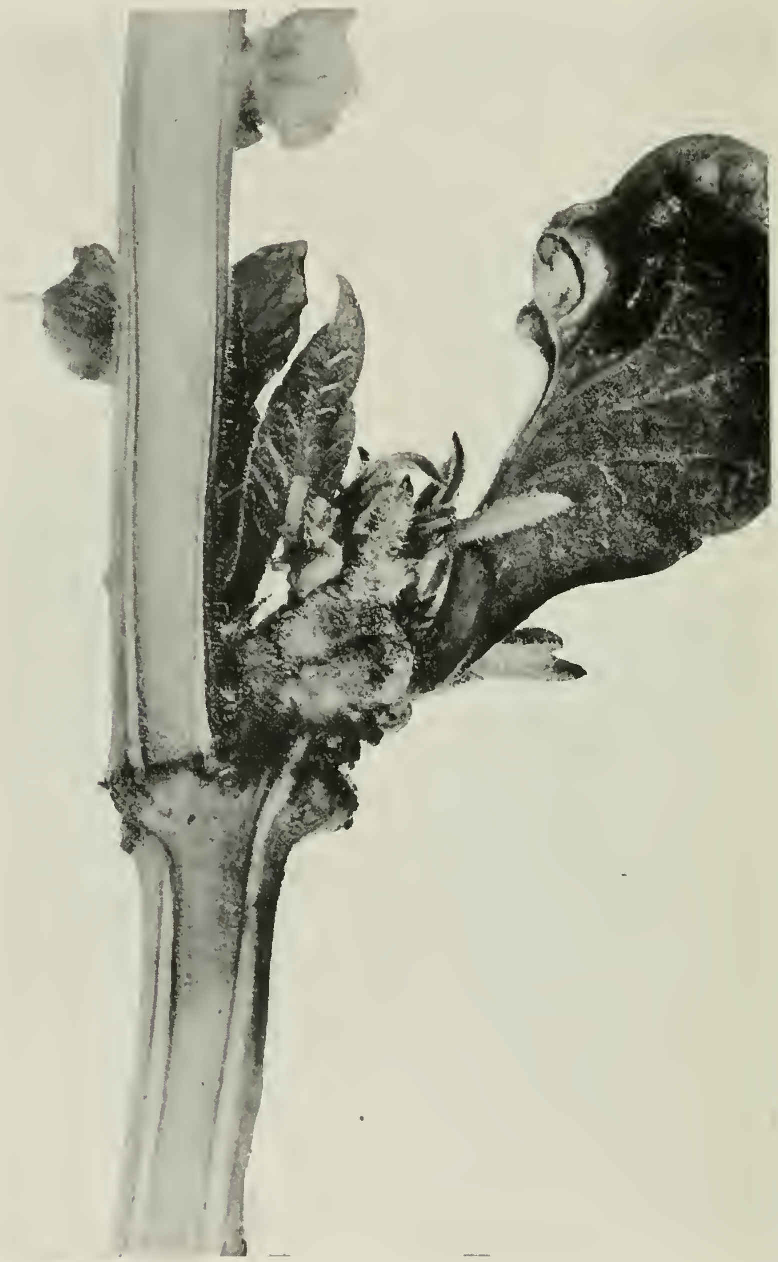

Fig. 9. Longitudinal Section of a Stem of a Tobacco Plant

The apical portion of the stem was removed and the cut surface inoculated. The crown-gall shows the development of leafy shoots.

DISCUSSION

The analysis of the material shows that crown-gall is undoubtedly a *neoplastic disease* and that the pathogenesis of the condition consists in an abnormal proliferation of a group of cells.

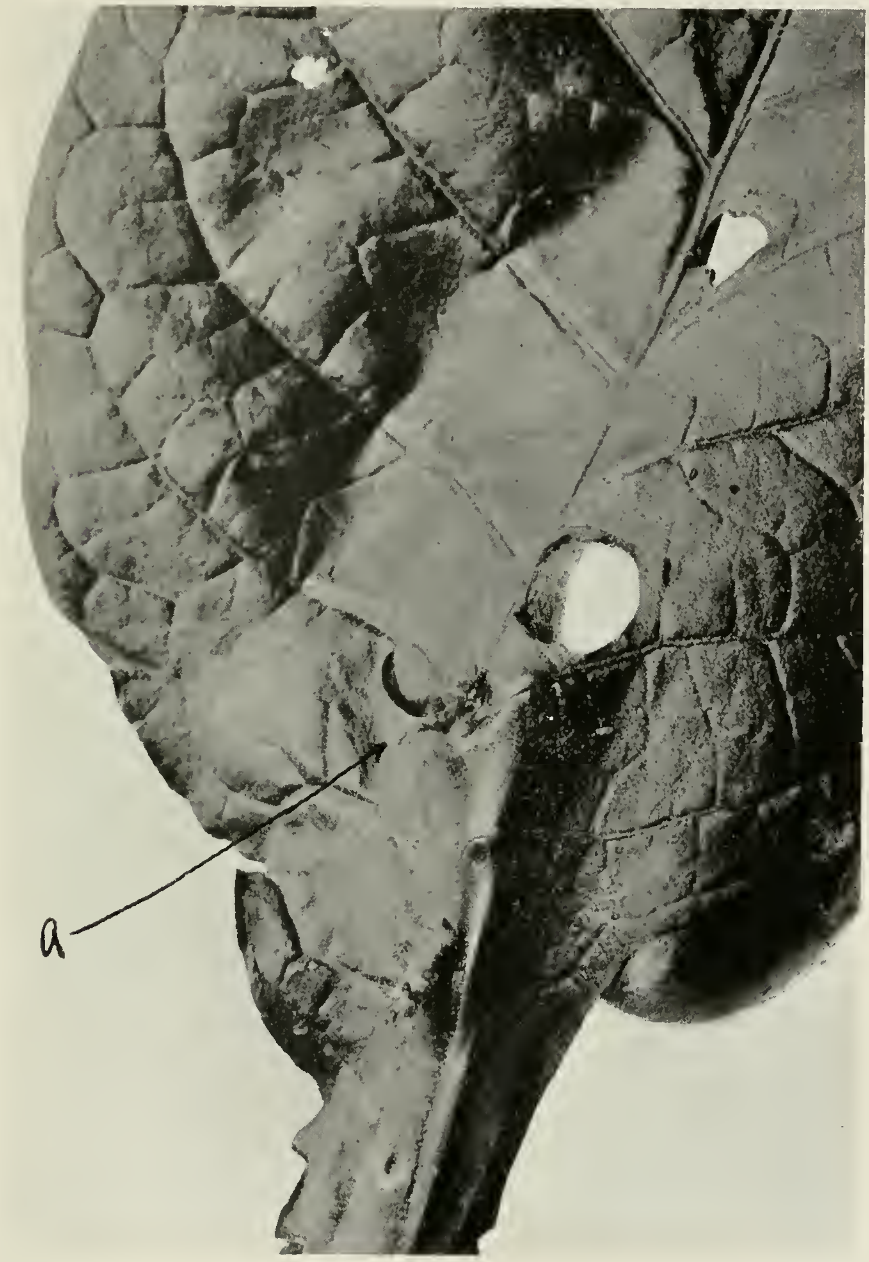

Fig. 10. Tobacco Leaf Inoculated at the Mid Rib
Shows at *a* a small gall with a leafy shoot

It is self-evident, therefore, that there must exist many points of analogy between the crown-gall and animal cancer. Since a plant presents a much less complex organization than the animal, crown-gall tissue undoubtedly offers an ideal material for the study of the phenomena connected with cell proliferation. All this does not imply that crown-gall or any other neoplastic disease in animal or plant must of necessity be a tumor, even less so a malignant tumor—a cancer.

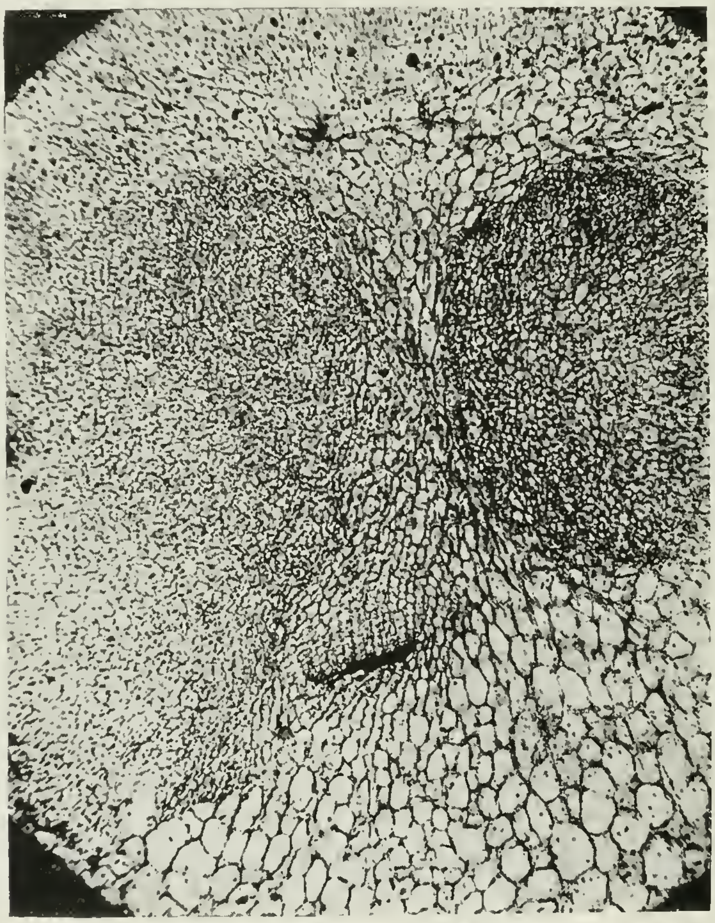

FIG. 11. MICROPHOTOGRAPH
Shows crown-gall tissue invading the normal stem tissue

In comparing new growth in animals and plants one must take into consideration the fact that an adult vertebrate is not capable of reproducing complete organs, while the highest plants may and do constantly reproduce with ease all their organs, leaves and branches as well as roots. Reproduction of parts of the organism, and consequently cell proliferation, is a function of an adult plant which may be induced with the greatest of ease.

It is self-evident that since an adult plant cell proliferates so as to reproduce an organ, a young crown-gall cell may do likewise. This characteristic of a normal plant cell explains the striking phenomenon, not encountered in any neoplastic disease in the animal, that a crown-gall may form within its own cells, or rather

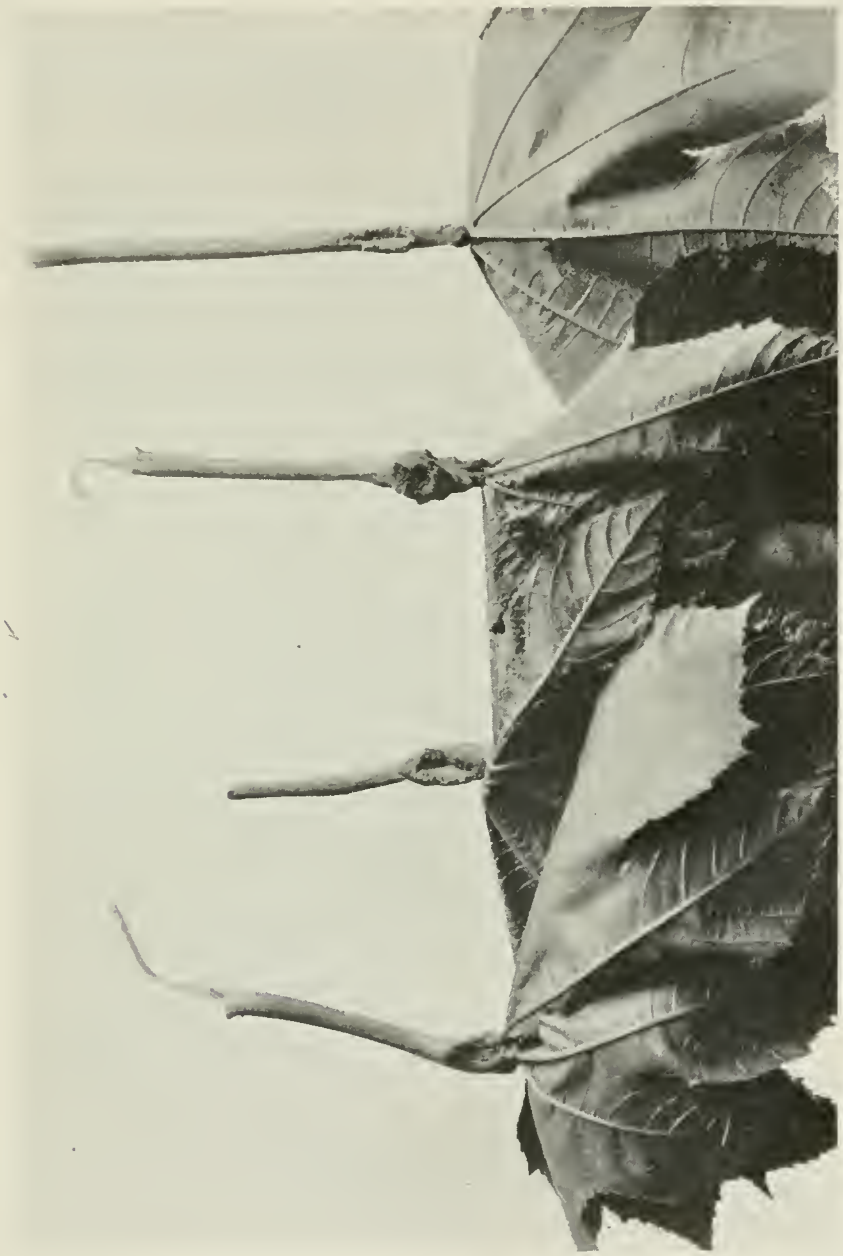

Fig. 12. Four Leaves of Ricinus Inoculated at the Upper Portion of Petioles
Shows small wart-like galls

as a transformation of its cells, not only adult differentiated tissues (parenchyma) but even rudimentary organs (conducting system) or a whole rudimentary organism (leafy shoot). All these various types of structures found in a crown-gall do not indicate, as appears to be the opinion of Smith, that the crown-gall is analogous to all the types of human cancer, but rather that it is different from any type of animal tumor.

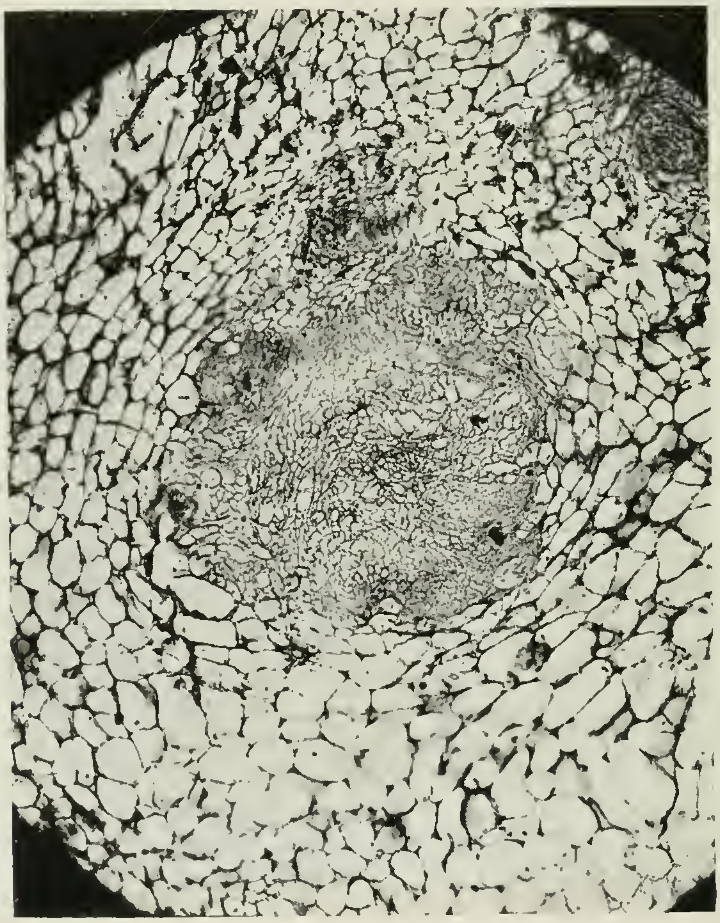

Fig. 13. Microphotograph of a Gall Shown in Figure 12

The characteristic gall tissue is surrounded by newly formed parenchyma tissue.

In order to formulate clearly the position which the crown-gall occupies among the neoplastic diseases, one must take into consideration the fact that the crown-gall is usually a benign condition and only rarely does it act in a manner analogous to a malignant tumor in an animal.

The true mechanism of the formation of a benign crown-gall can be conceived only in the light of the difference in the structure of an animal and a plant organism. An animal organism reacts frequently to an attack of any injurious agent with the so-called inflammatory processes, which are accompanied by certain activities of the lymphoid tissues and proliferation and migrations of lymph cells. These cells, and not the special tissues of the injured region, offer protection, envelop and destroy the injurious agent, or neutralize it. When the latter is destroyed, the inflammatory process ceases and functions of repair or replacement of the injured tissue take place. This repair, which ends in the formation of a scar, is a type of neoplasia which differs radically from tumor formation. It continues just as long as is necessary to replace the lost tissue and then ceases; while tumor formation is a neoplasia which has no reason for its formation in the needs of the organism, and has no finality in its development.

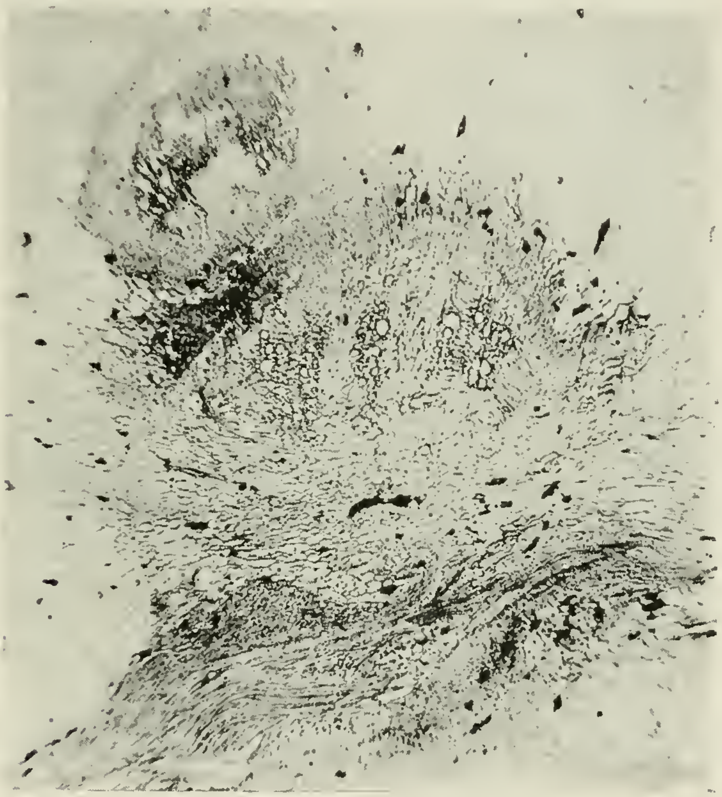

Fig. 14. Microphotograph

Crown-gall showing newly formed irregular conducting system

The highest organized plants do not possess any specialized lymphoid tissue to take care of the functions of the protection of the organism against an injury or of the repair of the injured or lost tissue. One of the methods of protection of plants against injury consists in the presence of a cellulose wall. It seems plausible to suppose that as a matter of self-protection a plant may respond to an injury by a proliferation of the cells of the region which was subjected to the injury. The small wart-like galls, which grow very rapidly for a time and then remain stationary, as the writers have observed in a number of instances, are products of such functions of repair and protection and do not represent true tumors. That the Bacterium tumefaciens may be found even within the crown-gall cells or in the immediate neighborhood, still further emphasizes the truth of this conception.

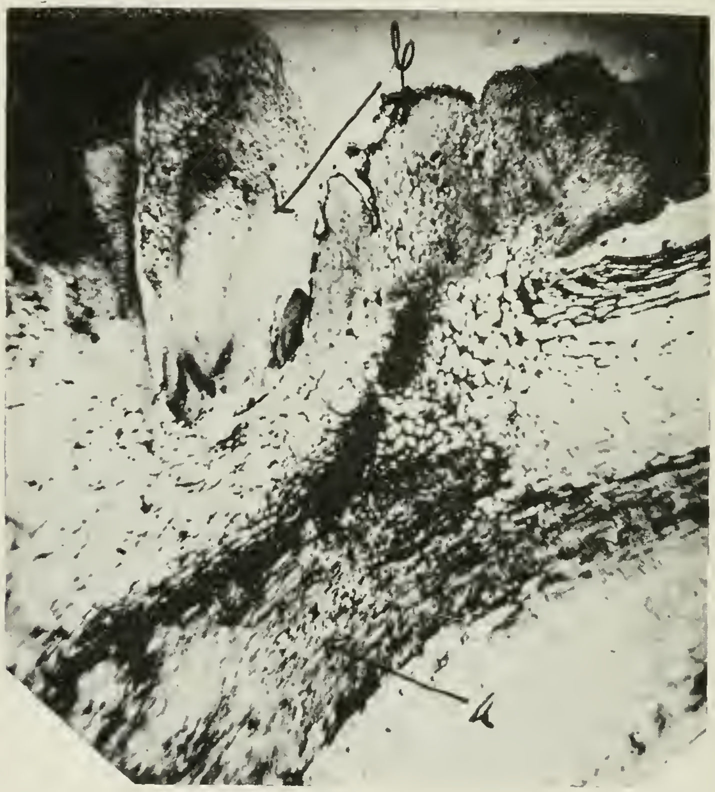

Fig. 15. Microphotograph
At *a* crown-gall tissue, and at *b* the tissue of a leafy shoot

Thus a small benign crown-gall is a condition analogous to granulation tissue and a scar in the animal organism. A crown-gall which grew to comparatively large size and still does not affect in any way the general welfare of the plant may be compared to a benign tumor in an animal, but is probably more nearly analogous to a large callus, which develops after a fracture of a long bone, or to a cheloid in the human.

Smith ascribes a great deal of importance to the phenomenon of development of leafy shoots in a certain number of crown-galls, and considers this type of gall to be identical with human embryoma. This finding he considers the main proof for his contention that all types of human cancer may be reproduced in plants by the aid of the Bacterium tumefaciens. In the opinion of the writers there is no analogy between a human embryoma and the crown-gall with a leafy shoot sprouting from it. An embryoma is a growth consisting of an irregular combination of various fetal tissues. It is more akin to a malformation than to a true tumor. A malignant tumor may develop subsequently within an embryoma in the same manner as it develops within normal tissue. The leafy shoot on the other hand appears on a fully developed crown-gall and is identical with a shoot which develops in a normal part of a plant. It simply indicates that crown-gall tissue as well as normal plant tissue may reproduce complete organs and thus still more widens the gap between the animal tumors and the crown-gall.

The malignant type of crown-gall is undoubtedly quite analogous to animal cancer. This condition occurs very rarely, as is seen from the results of the field studies on sugar beets of Townsend, who found that the destruction of the beets by the gall is not sufficient to influence the tonnage of the crop.

The most enthusiastic supporters of the parasitic theory of cancer admit that the formation of benign tumors cannot be due to a parasite. In other words, they admit that the mechanism of the formation of benign and malignant tumors must differ from each other. The same is undoubtedly true as regards the formation of the usual benign type of crown-gall and the by far less frequent condition when the gall dwarfs or destroys the plants and thus acts as a malignant tumor. When two plants of the same size, the same age, growing in the same soil under identical conditions of heat, light, moisture and nutrition, are inoculated in the same regions with the same quantity of an identical culture of Bacterium tumefaciens, and there develops in one plant a small, benign, wart-like structure and in the other a large malignant tumor which may destroy the plant, it is difficult to conceive that the same microorganism, and only the microorganism, created the two conditions. The only possible explanation of the phenomenon lies in the fact that in the second plant, for some unknown reason, the cell proliferation which began only as a protection against the bacterial invasion and apparently ceased when the bacteria were rendered harmless, suddenly received an impetus for limitless proliferation. It seems then that the first impetus to the cell proliferation and formation of a benign crown-gall may be caused by the Bacterium tumefaciens. But the transformation of this protective or inflammatory benign cell proliferation into a malignant tumor is due, as in every type of animal and human cancer, to some mechanism within the organism of the host, independent of the microorganisms, the nature of which is unknown.

CONCLUSION

1. Crown-gall is a neoplastic disease, and offers an ideal material for the cytological study of cancer.
2. Crown-gall occurs both as a benign and a malignant condition.
3. The benign crown-gall is analogous to granuloma or cheloid in the human and is caused by Bacterium tumefaciens.
4. The malignant crown-gall is analogous to animal cancer, and Bacterium tumefaciens is not the direct cause of the malignant transformation.

ON THE KINETIC AND INVASIVE POWER OF REGENERATING TISSUE AND ON SIMILARITIES IN THE BEHAVIOR OF THYROID TRANSPLANTS AND CARCINOMAS

LEO LOEB

Received for publication, December 22, 1919

The following observations are of interest because they clearly demonstrate that some of the fundamental characteristics of cancerous growth can be found in regenerative growth. In addition, they bring out very sharply some of the factors which, under ordinary conditions, restrain regenerative proliferation.

I. INVASION OF BLOOD VESSELS BY REGENERATING THYROID TISSUE

In the course of a study of the factors which determine regeneration of the thyroid gland and the fate of thyroid transplants, we compared autotransplantation of one thyroid lobe in cases in which the second lobe had been left intact and in which it had been extirpated previous to the transplantation. We also compared the fate of autotransplants and homoiotransplants at various stages.

In an animal, a female guinea-pig (no. 758) weighing 370 grams, we transplanted one lobe of the thyroid subcutaneously into the same animal. The lobe of the other side was left intact. Seven days later the transplant was taken out for examination. The recovered piece was relatively large and cut into serial sections. Microscopic examination showed the following:

A connective tissue capsule rich in fibroblasts surrounds the piece; it encircles a ring of acini, which, however, is not complete, being interrupted at such places where fat tissue constituted the periphery of the transplant. The peripheral acini are the largest ones; they contain often well formed colloid. Other acini are filled with blood and a few contain some polynuclear leucocytes. Towards the center the acini become smaller, and especially is their lumen diminished. At various places the inner acini seem to end in cell strands without lumen. These cell strands accompanied by growing fibroblasts grow towards the center of the piece, which latter consists in the main of thyroid tissue which, being deprived of blood supply after transplantation, had become necrotic and in part hemorrhagic. In the necrotic walls of the central necrotic acini some disintegrating polynuclear leucocytes are seen. In one small area of the necrotic center a somewhat larger collection of disintegrating polynuclear leucocytes can be observed. The acini as well as the more centrally situated epithelial strands contain relatively frequent mitoses. In the peripheral concentrically arranged capsule of connective tissue we find some thyroid tissue in the form of extensive ducts; they may send off branches in various directions. On the whole they follow the direction of the connective tissue capsule in which they are embedded. These ducts are perhaps acini which regenerated in the connective tissue capsule, and under the influence of the growing connective tissue assumed a concentric growth similar to that of the connective tissue itself. Mitoses are also found in these ducts. They are situated in the periphery of the ring of acini. There is on the other hand a possibility that they took their origin in epithelial ducts which occur even in the normal thyroid.

In the periphery of the transplant and inside of the connective tissue capsule we find running parallel with the capsule a large vessel. It had been transplanted with the thyroid and may perhaps have partly recovered after transplantation. It consists of two layers, an inner endothelial and an outer muscle layer. It is situated at a place where the thyroid ring is interrupted by fat tissue. This vessel gives off branches which enter septa of the transplanted thyroid and begin to take a course radially towards the center.

At one place the vessels connect with a large capillary which, accompanied by fibroblasts, traverses the greater part of the

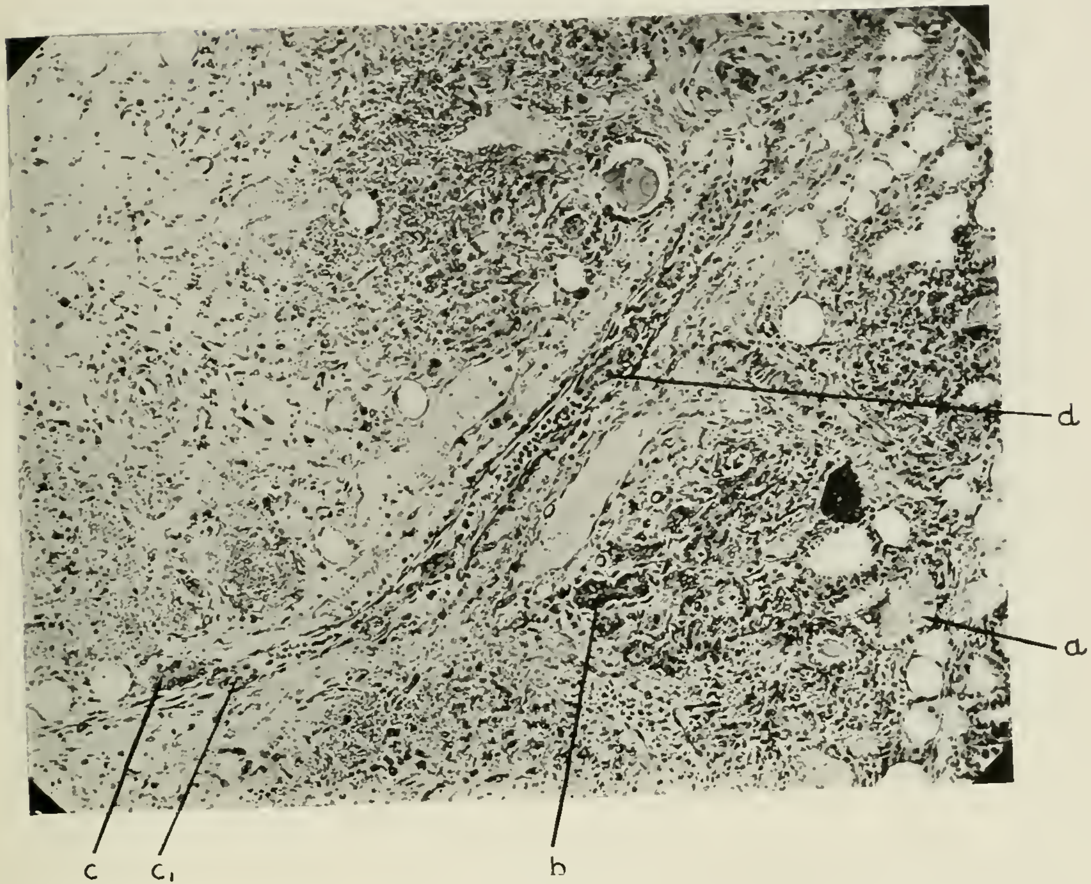

FIG. 1

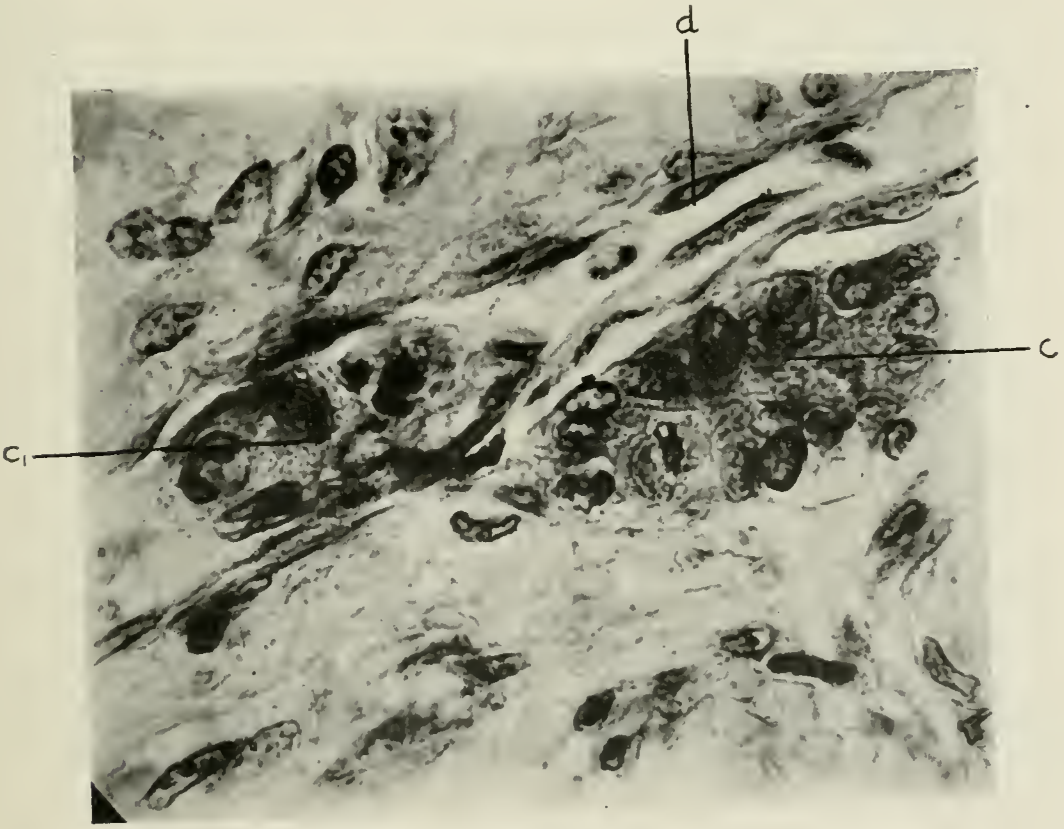

FIG. 2

necrotic center of the thyroid and here ends as narrow capillary tubes. This capillary again sends off branches at various places. There is actual growth taking place in these vessels, as evidenced by the presence of mitoses in the endothelial cells. It is furthermore probable that the coat of unstriated muscle cells which surrounds the larger vessels gradually extends towards the periphery, certain pictures suggesting that migration of cells may play a part in this growth process.

Fibroblasts accompany the growing vessels and organize the necrotic tissue which the vessels traverse. These fibroblasts gradually invade more and more the necrotic center and would in time have organized it.

At several places solid strands of thyroid tissue which are arranged in a radial centripetal direction grow towards the end ramifications of the capillary vessel. At two places they come in close contact with it. At one of these two places we see parts of the thyroid strands outside of the vessel but in close approximation to it. At one place such a strand breaks through the capillary wall and enters the lumen of the vessel (figs. 1 and 2). And from here on one can follow in serial sections how these strands of thyroid tissue fill the greater part of the lumen of the capillary, then enter the large peripheral vessel which we described above (fig. 3) and which connects with the capillary; at last they enter into the various branches of this vessel (fig. 4). Altogether considerable masses of newly formed thyroid tissue fill these vessels. Active proliferation continues in these strands within the blood vessels. We could find several mitoses in the thyroid cells. While in the capillary the thyroid tissue forms thin strands (fig. 1), in the larger vessels it is present in wider aggregates of tissue in accordance with the greater width of the vessels at the periphery of the thyroid (figs. 3 and 4).

While near the place of entrance into the capillary vessel the strands of thyroid tissue are lying free in the lumen, a little higher up they are covered by a layer of endothelial cells which are derived from the endothelium of the vessel. Wherever the thyroid strands are in touch with the vessel wall, endothelium begins to migrate over the strange tissue; thus in the course of time a cer-

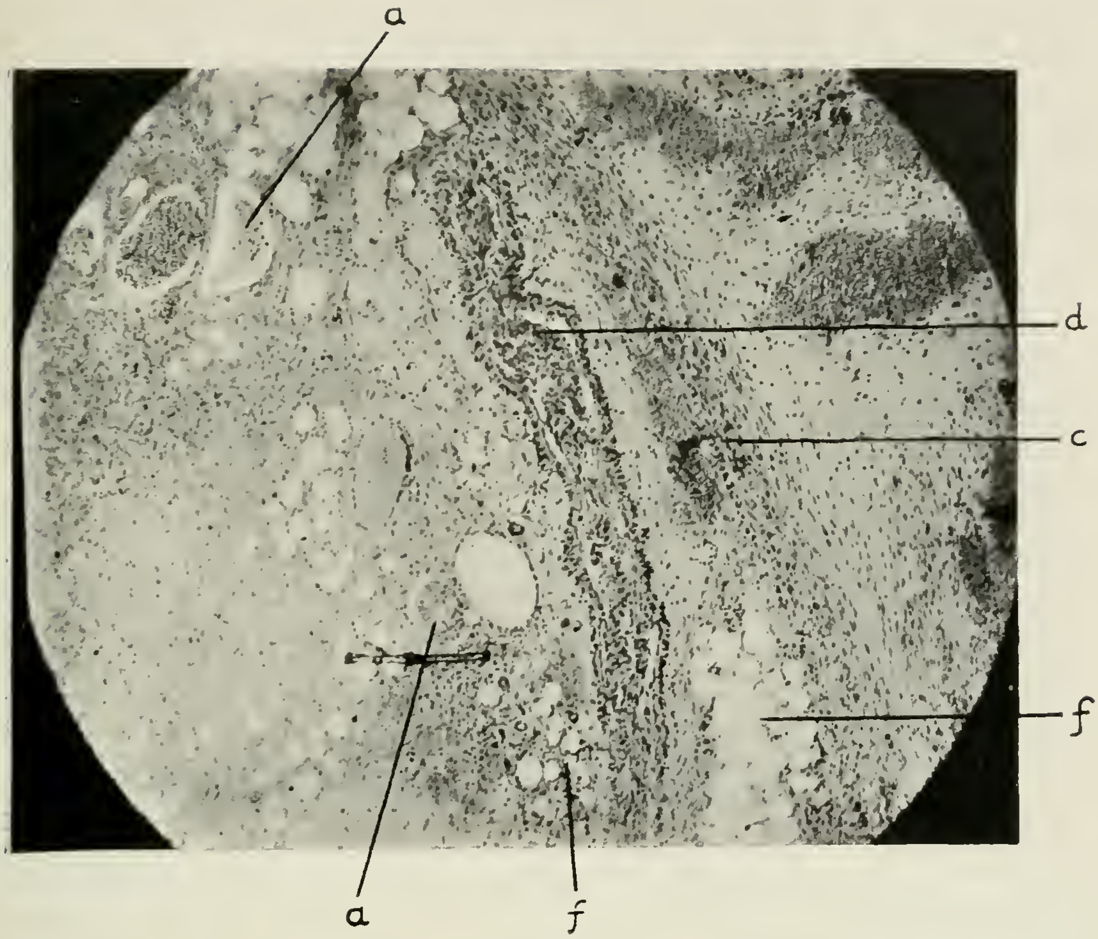

FIG. 3

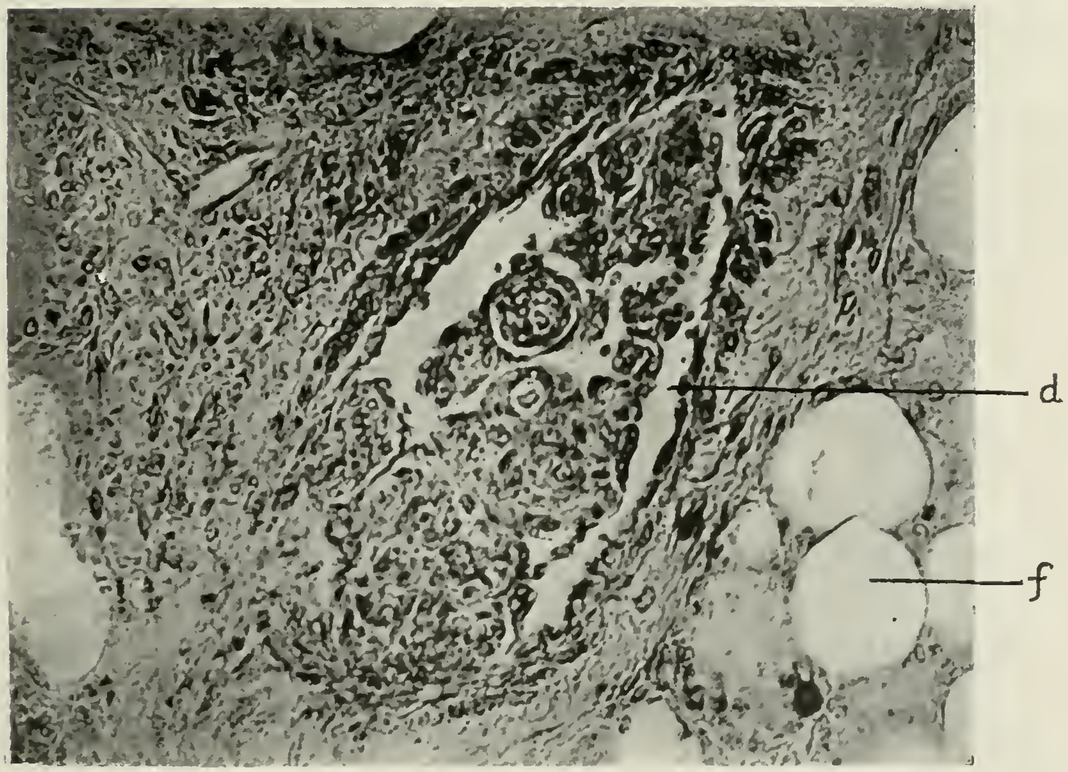

FIG. 4

tain inhibiting effect will be exerted upon the invading tissue by the endothelium of the vessel wall. This inhibiting influence is however as yet unable to prevent mitotic proliferation. More towards the periphery of the transplant within the larger vessel, the thyroid strands form irregular gyrations which are all covered by an endothelial coat. Between these strands of thyroid tissue within the larger vessels spaces are left in which the blood is able to circulate. We see here considerable masses of well preserved blood corpuscles. Further toward the center of the transplant in the end piece of the capillary we find mainly polynuclear leucocytes. Here evidently stagnation in the blood current occurred and in consequence of this the white corpuscles collected in larger numbers. A similar aggregation of polynuclear leucocytes occurs, whenever the circulation is slowing down at a certain place.

We must assume that at least temporarily the invading tissue has come to a standstill within the vessel; otherwise an investment with endothelium would not have been possible; nevertheless it is very probable that a shifting of the tissue in a peripheral direction continued to take place as a result of mitotic proliferation and perhaps also of continued invasion in the capillary in the center of the transplant. Furthermore considering the presence of circulating blood within the larger vessel a breaking off of certain parts of the thyroid strands is a possibility. Such broken off tissue might be carried into capillary vessels of distant organs and perhaps remain alive for a certain period of time.

II. INVASIVE AND GROWTH POWER OF REGENERATING AND CARCINOMATOUS TISSUE

These observations are of general interest in the first place because they demonstrate the marked similarity between regenerating and cancerous tissue, and secondly because they prove that the potentiality of forming new tissue inherent in regenerating tissue is in many cases considerably greater than could be assumed from the amount of tissue which is actually formed.

Cancerous tissue shows an increase in proliferating energy over the normal tissue from which it is derived; it likewise very often shows an increase in ameboid activity. As the result of both these processes an invasion of the surrounding tissue takes place as well as an invasion of blood and lymph vessels with subsequent formation of metastases. The stimulus acting on the cancerous cells may be very strong or the condition under which the stimulus reaches the cells may be abnormal. These abnormalities find expression in certain morphological changes which we often find in cancerous growth, such as a simplification in structure, irregularity in mitoses and in the structure and size of nucleus and cell and amitotic division of nuclei.

None of these changes are however entirely characteristic of cancer. An increase in proliferative energy and ameboid activity is found in regenerating tissue. We have formerly shown (1) that regenerating tissue may likewise show an increase in its power of infiltrating neighboring tissue especially after the latter has become less resistant. Thus we saw regenerating tissue entering blood clots, coagulated blood serum, and agar, and branching within these media in various directions. We found it breaking through cartilage and surrounding particles of cartilage and carrying them along on their migration. We may presume that in this case the cartilage had been previously injured, perhaps through a temporary infiltration with leucocytes. It is known that a simplification of structure is often observed in regenerating tissue and under certain conditions morphological abnormalities may be found in regenerating tissue similar to those observed in cancerous tissue.

We now can add to these characteristics the invasion of blood vessels on the part of regenerating tissues and at least the possibility that such invading tissue may become detached from its connections with the surrounding tissue and be carried through the blood stream to a distant organ. Whether here a real metastasis could ever be formed under these conditions appears doubtful considering the transitory character of the increase in proliferative energy which is characteristic of regenerating cells. The possibility can, however, not be denied that under certain conditions an additional stimulus, such as might perhaps be exerted by the abnormal environment, in which the transferred cells find themselves, may cause a retention of their increased growth energy and ameboid activity at least for a certain period of time, and that thus a metastasis consisting of apparently normal thyroid tissue may occur. Metastases of apparently not cancerous thyroid tissue have been noticed by various observers.

In a number of cases it has been observed that particles of apparently normal parenchyma entered the blood vessels and were retained in small blood vessels of the lung. Thus particles of bone marrow, liver cells and chorionic villi have been found (cf. O. Lubarsch (5)). There can be little doubt that in such cases we have to deal with a mechanical misplacement of tissue usually due to trauma of some kind. In no case is such an embolism the result of an active invasion of blood vessels such as we have observed.

The difference between regenerating and cancerous tissue is then mainly one which is quantitative as far as the intensity as well as the duration of changes are concerned, in cancer the increase lasting considerably longer than the stimulus and often becoming at least potentially perpetual or eternal. As a result of the action of proliferative stimuli a new cell equilibrium has been established in which through inner cell mechanisms those proliferative stimuli are furnished which originally had been called forth by external stimulation.

We see then that cancerous as well as regenerating cells are stimulated cells and, both show therefore similar reactions. The differences between regenerating and cancerous cells are mainly of a quantitative character as far as the energy and time factor are concerned and we must assume that in the cancer cells secondary inner regulations have taken place which are absent in the normal regenerating cells.

Our observations indicate that the amount of tissue which is actually produced during regeneration is considerably less than the amount which could be produced if certain restricting influences were absent. Thus we find that of the numerous cell strands which form at various places at the inner circumference of the thyroid ring only those situated at one or perhaps two places are able to produce large amounts of tissues, viz., those situated in close approximation to growing blood capillaries. They break into the vessels and encountering in the lumen of the vessel less resistance than outside, they extend over a considerable area partly through mitotic proliferation and in all probability also through cell movements. If other cell strands had been situated equally favorably near growing capillaries, they likewise might have penetrated into the lumen of the vessels and produced much new tissue. Then the total amount of newly formed tissue would have greatly exceeded the amount actually produced.

At all other places the strands found mechanical obstacles to their proliferation and extension. The necrotic and hemorrhagic material filling the center of the transplant and at some places perhaps also fibrous tissue opposed the epithelial growth. The expansive energy of the regenerating epithelium can evidently be neutralized by a certain amount of resistance which depends upon the mechanical properties of the environment. It is certain that the expansive energy of carcinomatous tissue being lastingly increased would be able to overcome a resistance which proves too strong in the case of regenerating tissue. Moreover in many cases the expansive power of carcinomatous tissue is not only extending over a longer period of time but is also more intensive. This is, however, not necessarily so in every instance. Thus we found in a case of multiple carcinoma of the skin in a young man the corium offering so strong a resistance to the expansive tendency of the cancerous tissue that at one place the latter was forced to proliferate towards the outside of the skin (2).

III. INVASION OF MUSCLE AND FAT TISSUE BY REGENERATING THYROID

These observations concerning the kinetic and invasive power of regenerating thyroid tissue do not represent exceptional or isolated occurrences. Thus we have found another transplant in which the regenerating acini contained in their lumen particles of fat tissue. They evidently had surrounded cells of fat tissue which were situated near them and closed around them to form acini.

Very frequently remnants of epithelial ducts or perhaps also peripheral acini which are situated in the growing fibroblastic tissue proliferate with the latter and form extraordinarily large epithelial spaces, which run parallel to the connective tissue. They may branch in various directions towards the central thyroid tissue as well as towards the surrounding proliferating connective tissue, and often multiple and large papillae are produced in these ducts as the result of the fibroblastic proliferation. We have mentioned such a duct in the first thyroid which we described in this paper.

In another case in which a thyroid had been removed seven days following homoiotransplantation (guinea-pig 670) we saw growing acini running parallel to proliferating connective tissue and advancing towards the outside and infiltrating the muscle tissue of the host; during this process the acini were accompanied by growing connective tissue and capillaries. At one place the lumen of an acinus contained a piece of the muscle tissue which had been surrounded by the growing thyroid strands and included in the acinus in a similar way to the fat tissue mentioned above. Many mitoses were found in this proliferating thyroid tissue.

IV. ACINAR AND ALVEOLAR TYPE OF GROWTH IN THYROID AND CARCINOMA

There is another similarity between the regenerating thyroid and certain carcinomata of the mammary gland in mice which however is perhaps in part at least more apparent than real. It is a very common occurrence that such carcinomata are structurally of a mixed character and of special frequency is a combination of acini in certain areas of the carcinoma and of solid alveoli in others. The alveoli often show a marked mitotic activity and they may thus enlarge; but it can be shown that there is still another mode in which alveoli grow; the apparently simple alveoli are in many cases merely conglomerations of cell strands, which form gyrate structures. Gradually as the result of the pressure, exerted by the epithelium, intervening areas of connective tissue become smaller, form a more or less homogeneous mass and ultimately disappear, and thus we have in the end to deal with apparently homogeneous alveoli, whose mode of origin from cell strands is no longer discernible.

In the regenerating thyroid several authors mention a formation of structures not unlike cell nests consisting of squamous epithelium. It is assumed that such cell nests are derived from proliferating acini. If this interpretation were correct, we had in this case to deal with a process analogous to what apparently occurs in carcinoma, viz., a transition from acinar to alveolar structures. Our observations make it however very probable that such a transition is not the mode or at least not the only one in which these alveolar structures originate. We found in the normal thyroid of the guinea-pig ducts which may branch and are surrounded by dense fibrous tissue. Even in the not transplanted thyroid such ducts may occasionally produce solid cell nests. The production of such cell nests from ducts seems to be a frequent occurrence after transplantation of the thyroid. The ducts accompanied by proliferating fibroblastic tissue grow actively and produce a very widely spread, branching network of solid strands, all connected with each other. The connective tissue around them becomes fibrillar and fibrous. In several cases we have been able to trace such a network of alveoli towards a central duct surrounded by dense fibrous tissue, such as we find in the normal thyroid. There can be very little doubt that in these cases the ducts and not the acini give origin to the alveolar structures. The process is analogous to what I have observed in the transplantation of mammary gland where also similar alveolar structures occur, and where they do not owe their origin to a transformation of gland acini, but to a proliferation of the excretory ducts of the gland. Thus all these alveoli are connected with each other.

It is possible that the alveolar structures found in the mammary carcinomata may also be derived from gland ducts rather than from acini. If this should be so, the similarity between the behavior of regenerating thyroid and certain carcinomata would be great. If on the other hand the alveoli of the mammary carcinoma are derived from acini, then the similarity would be in part at least more apparent than real. However at present we do not wish to exclude the possibility that the cell strands which take their origin in thyroid acini may also be able to produce solid alveolar instead of acinar structures under certain conditions. Thus in the lumen of the blood vessels the proliferating cell strands, which we described above, proliferate as solid strands and not as acini. It seems however probable that in most cases the typical network of large alveoli sometimes showing approximation to pearl formation is derived from ducts and not from acini.

In another respect however a certain similarity between the alveoli of mammary carcinoma in mice and the alveoli in the regenerating thyroid may exist. The former, as we have seen, may be composite structures. The same may hold good in the case of thyroid alveoli. Solid cell strands growing from different directions towards each other meet, include at first parts of the stroma, which later disappears. This condition was for instance observed in guinea-pig 499 in which a homoiotransplantation of a lobe of thyroid had been carried out eight days previously. It was a rather young guinea-pig, weighing 427 grams. There was found in this case a noticeable infiltration with lymphocytes and much hemorrhage in the center which was perhaps in part due to the rupture of newly formed capillaries. In the center we found solid alveoli. In serial sections it was seen that all or at least the majority of these strands were connected with each other (fig. 5). There is marked mitotic proliferation in these cells. This contributes to the enlargement of the alveoli, but in addition it appears that at certain places neighboring cell strands approach each other, and exert pressure on the intervening stroma and that thus processes of solution take place in the connective tissue. At first one may still be able to discern connective tissue strands within the apparently homogeneous alveoli but ultimately the strands disappear and then it is no longer possible to recognize the origin of these conglomerate alveoli. Simultaneously with these changes in the epithelial structures changes take place in the accompanying stroma. The fibroblasts form fibrillae and often dense fibrous tissue around these strands and alveoli and it may be that the changes from fibroblastic to fibrous tissue are in part responsible for the lessened nourishment and secondary changes in the center of the alveoli which correspond to similar changes which take place in the epidermis whenever it is further removed from the source of nourishment.

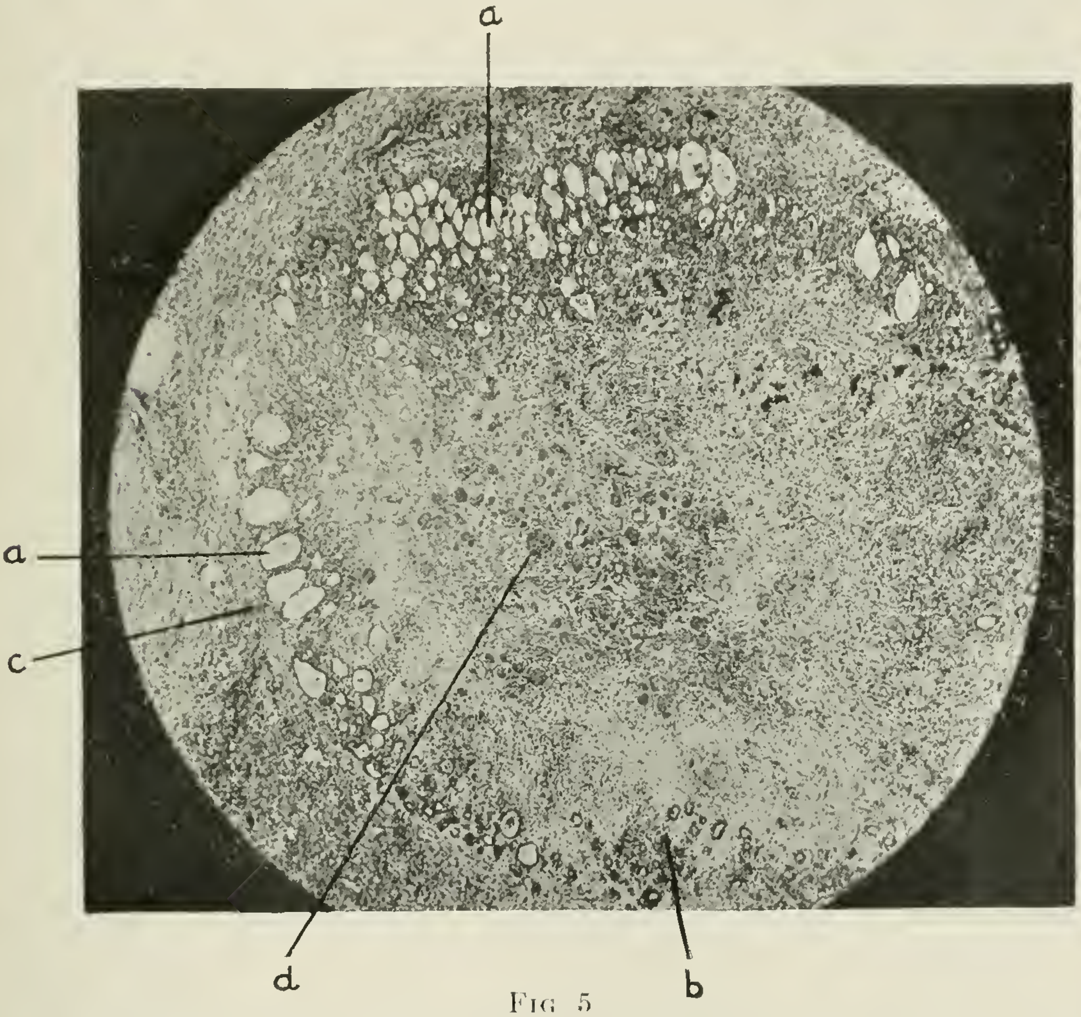

FIG 5

These solid cell strands grow especially towards the center and not within the peripheral ring of acini. This is evidently due to the fact that the fibroblasts grow from the peripheral ring towards the center of unorganized material which they organize. It is the movement of the fibroblasts which determines the direction in which the epithelial structures move.

These cell strands cannot under any circumstances succeed in reestablishing the normal thyroid structure. They will always remain epithelial tissue dissociated from neighboring normal tissue; they represent "cell nests." And yet growth within them ceases and they do not produce carcinoma. There is therefore one essential difference between regenerating and carcinomatous tissue. In the latter the growth stimulus continues to act independently of the environment in which the growing cells are, as long as the cells obtain sufficient nourishment; in regenerating tissue the stimulus leads to growth processes which can be represented by a curve which earlier or later approaches the base line, although normal conditions may not yet have been reestablished.

V. ON THE INTERACTION BETWEEN GROWING GLAND TISSUE AND FIBROBLASTS

In the cases mentioned above there can be little doubt that it it is the growing fibroblastic tissue which causes proliferation of the thyroid tissue and at the same time determines the direction in which the epithelium proliferates and moves. It determines the shape of the thyroid cell strands, and the degree and direction of their proliferation in the center of the transplant. It likewise determines the proliferation and the structure of the concentric canals in the periphery of the thyroid and the secondary formation of papillae within these canals. In all those cases the proliferating fibroblastic tissue does not only not prevent, but even stimulates the epithelial growth; at the same time it influences the direction in which it takes place. In accordance with these observations on the beneficial effect of fibroblastic proliferation on epithelial growth we often notice that after transplantation of an epithelial tissue the most active proliferation takes place in that part of the graft which is in contact with actively moving and proliferating fibroblasts of the host, while that part of the transplant which is at a place more distant from proliferating fibroblasts is often not exhibiting any noticeable growth. In the latter case, however, we cannot be quite certain which is the primary process, the fibroblastic proliferation stimulating the epithelial growth in the graft, or the epithelial growth attracting and stimulating the fibroblasts. On the other hand in the observations to which we referred above there can be no doubt that the fibroblastic tissue is the primary factor.

While thus it is certain that fibroblasts may through their activity stimulate epithelial growth, the reverse effect also occurs. We have formerly described the difference in the character of the stroma in resting and in active mammary gland of the guinea-pig (3). In this case there is no doubt that the epithelial activity is the primary factor which stimulates secondarily the connective tissue. Growing epithelial tissue usually stimulates the surrounding stroma. A similar stimulating effect may be exerted by the growing parenchyma on the blood vessels. In growing carcinoma the proliferation of the epithelial structures quite commonly stimulates the activity of the fibroblasts and of the blood vessels.

While thus the activity of epithelial tissue stimulates fibroblastic and vascular activity in the area adjoining the epithelium, the latter possesses, as we have pointed out previously, the power to prevent the penetration of fibroblasts into the epithelial structure and the epithelial tissue may even actively restrict the fibroblastic growth (4). It seems that it is the "autosubstance" produced in the epithelium which has this power in the highest degree and that it is partly lacking, whenever homoiosubstances are produced through the interaction of transplant and body fluids of the host.

The interaction between epithelial and fibroblastic tissue is therefore evidently complex. Epithelial activity stimulates fibroblastic activity and fibroblastic activity may under certain conditions stimulate epithelial activity. The epithelium possesses mechanisms through which it restrains the invasive power of connective tissue. Under certain conditions epithelium loses to some extent this power (for instance, under the influence of homoiotoxins) and then the connective tissue exerts a destructive influence upon the epithelium. Fibrous tissue restrains the activity of the epithelium and metabolically inactive or pathologically functioning epithelium may favor the transformation of the surrounding fibroblastic into fibrous tissue.

SUMMARY

1. Regenerating tissue of the thyroid may possess an invasive power not unlike that of cancerous tissue. Strands of regenerating thyroid can invade blood vessels and advance and proliferate within their lumen. They may also invade fat and muscle tissue and include in the lumen of the acini particles of such tissue. This invasive power is less intense than that exhibited by very active carcinomata.

2. Growing transplants of thyroid tissue may apparently show a transition from acinar to alveolar structure. Similar transitions can be found in the case of mammary carcinoma in mice. In both cases the formation of alveoli may be due to a conglomeration of cell strands. At first parts of stroma are still included between the epithelial constituents. Secondarily these become dissolved and disappear. It can be shown that in many cases the alveolar network in the center of the transplanted thyroid does not take its origin in acini, but in ducts included in the thyroid. In a similar way alveolar structures in transplants of the mammary gland originate in ducts.

3. The kinetic and invasive activity of regenerating thyroid tissue is associated with fibroblastic activity. There are indications that under certain conditions epithelium and connective tissue exert a mutually stimulating influence, while under other conditions their effect upon each other is antagonistic.

4. The power of regenerating tissue to proliferate is much greater than could be foreseen from the amount of tissue which is actually produced. Usually the environment exerts a restraining influence upon the regenerating activity of the transplant.

5. In contradistinction to carcinomatous growth regenerating growth of the thyroid comes to a standstill, although the typical structures of the thyroid may not yet have been reëstablished and the cell strands are as yet without their normal connections with neighboring epithelium.

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THE GLYCEMIC REACTION IN ITS RELATION TO TRANSPLANTABLE MALIGNANT TUMORS

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That hyperglycemia of a temporary character follows the injection of a relatively large variety of substances has been shown by the results recorded in another publication (1). The relation of this type of hyperglycemia to transplantable malignant tumors in animals forms the subject of the present paper. In the publication to which reference has been made, the observation that a hyperglycemia followed the injection of peptone was confirmed. Three types of reactions were described as occurring after such injections. It was demonstrated that there was no constancy about the reaction type, irrespective as to whether the same or different substances were used in repeated injections.

The first experiment recorded in the present paper has to do with the constancy of the reaction type after the growth of transplanted tumors.

A series of rats was injected with 0.125 cc. of a 1 per cent solution of peptone in distilled water. Immediately before injection and again forty-five and one hundred and twenty minutes thereafter, blood sugar estimations were made according to the Epstein (2) method, blood being obtained from the tail vein.[1] The animals were then inoculated with 0.003 gram of either the Flexner-Jobling carcinoma or the Jensen sarcoma. After twenty-one days of tumor growth the animals were again tested with peptone as before inoculation. As controls, a small series of

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[1] In the four animal experiments, all blood sugar determinations were made after starving the animals for twelve hours.

TABLE 1

All figures indicate milligrams of sugar per 100 cc. of blood

	BEFORE				AFTER			
	Zero hour	45 minutes	120 minutes	Type	Zero hour	45 minutes	120 minutes	Type
Flexner-Jobling carcinoma; tumor growing....	180	220	248	I	152	176	160	II
	140	204	212	I	168	174	200	I
	122	120	110	III	140	244	164	II
	110	208	188	II	120	182	168	II
	176	168	200	III	136	110	130	III
	186	158	160	III	152	162	160	II
	108	120	150	I	110	120	124	I
	124	130	142	I	114	110	134	III
	124	144	170	I	130	142	162	I
	138	228	200	II	120	124	130	I
	130	136	134	II	130	160	200	I
	130	140	178	I	140	144	189	I
	156	174	200	I	130	164	108	II
Flexner-Jobling carcinoma; tumor not growing	190	186	174	II	134	130	146	III
	222	176	110	III	110	152	140	II
	124	130	160	I	128	104	162	I
	116	200	124	II	138	164	200	I
	140	132	140	III	134	120	132	III
	170	140	180	III	180	210	160	II
	100	108	136	I	154	178	164	II
	132	178	130	II	110	140	176	I
Jensen sarcoma; tumor growing...............	102	198	150	II	120	160	168	I
	240	194	168	III	124	148	180	I
	156	150	114	III	130	136	150	I
	140	126	142	III	160	130	138	III
	144	146	134	II	144	190	186	II
Jensen sarcoma; tumor not growing...........	146	170	184	I	138	130	136	III
	156	122	114	III	170	172	128	II
	150	144	140	III	126	104	100	III
	130	160	116	II	132	146	132	II
Fetal skin emulsion......	102	90	110	III	110	140	142	I
	116	100	86	III	120	160	154	II
	138	100	180	III	112	159	154	II

animals were tested with peptone as were the other animals, and then injected with 0.05 gram of emulsified fetal rat skin. These animals were also retested with peptone twenty-one days after the injection of the fetal skin.

The data of this experiment, which are given in table 1, show that, as was to be expected, no constant type of reaction occurred before inoculation with tumor or injection with fetal skin, and that twenty-one days after the introduction of these cells, the same conditions of affairs existed, irrespective as to the fate of the transplanted graft.

Other experiments carried out concurrently with those described in this paper having indicated that the hyperglycemia following the injection of substances other than glucose was probably related to the processes of immunity, our next experiments were planned so as to use hyperglycemia as an indicator of the development of antibodies, in order to see if hyperglycemia, when interpreted in this fashion, would follow the laws so far determined of immunity against transplanted animal tumors.

It is generally accepted that immunity to transplanted tumors can be induced by the injection of homologous living cells at least ten days before tumor inoculation. It is also generally accepted that autologous or heterologous living cells do not have this power (3).

In one experiment, rat fetal skin was emulsified and extracted for three hours in distilled water. Then 0.125 cc. of this extract was injected subcutaneously into a series of normal mice, spontaneous tumor-bearing mice, and mice bearing the transplanted Crocker Fund mouse carcinoma no. 11. As in our rat experiments, blood sugar estimations were made just before, and again forty-five and one hundred and twenty minutes after injection. The data given in table 2 show that rat fetal skin, representative of an heterologous protein, was capable of inducing a temporary hyperglycemia in all three groups. This result is comparable to the antibody production which follows the injection of any foreign protein, in that while a foreign protein induces antibody formation when injected, it also provokes a hyperglycemia.

In another experiment, the effect of injections of autologous protein extracts was studied. The spleens were removed from a group of rats, this operation having been shown in our first paper not to interfere with the hyperglycemic reaction. The spleens were emulsified and extracted in their own bulk of distilled water for three hours. As before, sugar estimations were made just before, and at the stated periods after, the subcutaneous injection of 0.125 cc. of this extract. In a similar manner extracts of spontaneous mouse tumors were prepared, and as with the spleen animals, each extract was injected into the animal from which the tissue was derived. In a third group of animals bearing transplanted Jensen rat sarcomata twenty-one days old, the tumors were removed, emulsified, and extracted,

TABLE 2

Effect of injections of heterologous protein extracts. All animals injected with 0.125 cc. of rat fetal skin extract. All figures indicate milligrams of sugar per 100 cc.

	ZERO HOUR	45 MINUTES	120 MINUTES
Normal mice	140	152	118
	170	140	110
	150	192	215
	128	135	165
	117	128	175
	131	190	110
Mice bearing growing mouse carcinoma 11	136	130	158
	147	156	120
	110	120	184
	174	150	192
	180	130	120
	117	130	142
Mice bearing spontaneous tumors	190	171	150
	210	141	230
	174	185	225
	185	171	148
	192	140	190
	165	173	196

as were the other tissues, the extract in turn being injected into the animal from which it was derived. The data of this experiment are presented in table 3.

TABLE 3

Effect of injections of autologous protein extracts. All figures indicate milligrams of sugar per 100 cc.

	ZERO HOUR	45 MINUTES	120 MINUTES
Normal rats injected with autologous spleen extract	170	172	172
	180	178	180
	168	165	164
	148	150	146
	160	159	157
	152	154	152
	148	148	150
	190	192	194
	165	161	160
	158	159	155
Normal rats injected with autologous Jensen sarcoma extract	150	152	150
	160	160	158
	220	140	142
	146	148	150
	195	124	210
	134	140	136
	170	140	195
	161	160	163
	157	157	158
	165	121	210
Normal mice injected with autologous spontaneous tumor extract	180	180	178
	164	166	170
	176	176	178
	200	200	200
	184	183	184
	190	192	188

Note that autologous protein extracts induce no reaction in normal animals or in spontaneous tumor-bearing animals; but that autologous transplanted tumors sometimes give rise to a reaction.

As is shown in that table, autologous spleen extract and autologous spontaneous tumor extract did not give rise to a reaction, or to put the matter more exactly, gave rise to a reaction so small (variations of 10 mgm. or less have arbitrarily been classed as no reaction) as to be within the factor of error of the method of determination. In contrast to this, extracts of autologous transplanted tumor sometimes gave rise to a reaction, and sometimes did not. While, broadly speaking, a transplanted tumor may be considered autologous, in a strict sense it is not, since it arises from a group of cells which several years ago were autologous to another animal. The present host merely furnishes a residence and nourishment, so to speak.

TABLE 4

Animals bearing transplanted tumors injected with autologous tumor extract. Sugar estimation made just before injection and again one hour after. Variations of 10 milligrams or less classed as negative glycemic reaction

TUMOR STRAIN	NEGATIVE REACTION		POSITIVE REACTION	
	Receding	Growing	Receding	Growing
Flexner	0	18 (75%)	0	6 (25%)
Jensen	6 (28%)	1 (4%)	13 (59%)	2 (9%)
Mouse 11	1 (13%)	7 (87%)	0	0

Average figures: Blood sugar

Flexner			438	490			461	467
Jensen	480	480	462	464	492	444	454	490
Mouse 11	444	456	512	515				

Summary. Of those animals, tumor strain not separated, which showed receding tumors, 65 per cent gave a glycemic reaction; while of those which showed growing tumors, 76 per cent gave no glycemic reaction.

A further investigation of the variations occurring in animals bearing transplanted tumors was undertaken. A number of animals were inoculated with the usual dose of either the Flexner-Jobling rat carcinoma, the Jensen rat sarcoma, or Crocker Fund mouse carcinoma no. 11. After twenty-one days of tumor growth, portions of the tumor were removed, and extracted in distilled water; and the extract, in dosage of 0.125 cc., was injected subcutaneously into the animal from which the tumor had been removed. Blood sugar estimations were made at the same time periods and according to the same method as in the previous experiments.

The summarized data are presented in table 4. Of those animals, tumor strain not separated, which showed receding tumors, 65 per cent gave a glycemic reaction, i.e., showed variations of more than 10 mgm. in the three estimations made. In contrast to these figures, but 24 per cent of the animals in which the tumor continued to grow gave a glycemic reaction.

The experiments so far recorded have shown that heterologous protein extracts are capable of inducing a hyperglycemia in normal as well as in spontaneous and transplanted tumor-bearing animals. On the other hand, autologous protein extracts do not induce a hyperglycemia in normal or in spontaneous tumor-bearing animals, though occasionally they may in animals bearing transplanted tumors.

In a final experiment, the effect of injections of homologous protein in normal animals, in transplanted tumor-bearing animals, and in spontaneous tumor-bearing animals was studied. In this experiment several Flexner-Jobling rat carcinomata, twenty-one days old, were emulsified and extracted with distilled water. In a similar manner extracts of Jensen rat sarcoma and of rat spleen were prepared. These extracts were injected in a dosage of 0.125 cc. into animals bearing the Flexner tumor, into those bearing the Jensen tumor, and into normal animals. Extracts of mouse carcinoma 11 and of mouse spleen were also prepared and injected in a dosage of 0.125 cc. into animals bearing spontaneous tumors. Blood sugar estimations were made as before at the stated time periods. The data which are given in tables 5 and 6 show a rather surprising result, in that homologous protein as exemplified by the extracts of either the Jensen or Flexner tumor or of rat spleen did induce hyperglycemia in normal rats, and in rats bearing either one of the two transplantable tumor strains, while animals bearing spontaneous malignant tumors in a large percentage failed to give hyperglycemia when injected with either homologous tumor or normal tissue.

Speculations based upon the results of the experiments recorded here are interesting. It is apparent that there is a biological difference between a majority of animals bearing spontaneous tumors and other animals, which difference is demonstrable by the failure of spontaneous malignant tumor-bearing animals to respond by hyperglycemia upon the injection of homologous protein extract. That some biological difference exists between normal and tumor-bearing individuals is suggested in many other ways; for example, by the clinical observation that an irritant known to produce carcinoma in some indi-

TABLE 5

Effect of injections of homologous protein extracts. All figures indicate milligrams of sugar per 100 cc.

	ZERO HOUR	45 MINUTES	120 MINUTES
Normal rats injected with Flexner tumor extract..	141 139 156 171	178 152 194 162	130 192 130 141
Normal rats injected with Jensen tumor extract..	132 148 151 146	155 185 132 121	175 196 190 184
Normal rats injected with spleen (rat) extract....	155 151 157 160	171 162 174 130	135 185 196 110
Rats bearing Flexner tumor injected with spleen (rat) extract..................................	144 164 176 190	160 150 168 160	130 100 150 130
Rats bearing Flexner tumor injected with Flexner tumor extract................................	148 162 156 160	124 120 160 118	162 140 132 124
Rats bearing Jensen tumor injected with Flexner tumor extract................................	170 148 161 136	160 175 195 152	110 195 130 183

TABLE 6

Glycemic reaction in animals bearing spontaneous tumors when injected with homologous protein extract (mouse spleen)

ANIMAL NUMBER	DIAGNOSIS	PREOPERATIVE	
		Zero hour	1 hour
83	Sarcoma of neck	320	328
84	Fibroma of breast	304	336
85	Adenoma of breast	440	404
1741	Carcinoma of breast	160	160
1746	Carcinoma of breast	150	148
1752	Carcinoma of breast	158	156
1755	Carcinoma of breast	176	178
1760	Carcinoma of breast	540	472
1761	Carcinoma of breast	460	520
1762	Carcinoma of breast	528	524
1763	Carcinoma of breast	504	504
1764	Carcinoma of breast	520	524
1765	Carcinoma of breast	416	460
1766	Carcinoma of breast	448	460
1767	Carcinoma of breast	440	504
1771	Carcinoma of breast	452	520
1772	Carcinoma of breast	408	408
1773	Carcinoma of breast	444	448
1774	Carcinoma of breast	476	476
1777	Carcinoma of breast	508	508
1778	Carcinoma of breast	452	448
1779	Carcinoma of breast	368	436
1781	Carcinoma of breast	540	540
1782	Carcinoma of breast	536	568
1783	Carcinoma of breast	500	500
1784	Carcinoma of breast	564	564
1787	Carcinoma of breast	524	524
1791	Carcinoma of breast	536	524
1792	Carcinoma of breast	532	532
1793	Carcinoma of breast	560	560
1794	Carcinoma of breast	545	548

Of 28 animals bearing spontaneous tumors, 75 per cent gave no glycemic reaction when injected with homologous protein. In the later two-thirds of this series 0.05 cc. of blood was taken for the sugar estimation instead of 0.2 cc. as in the previous experiments. It has been our experience that when such small amounts are used the values obtained are much higher than when larger amounts of blood are used.

viduals does not produce it in all. If the irritant were the sole factor, a neoplasm should follow in the greater proportion of all exposed to the same irritant. How long this biological difference exists in spontaneous malignant tumor-bearing animals before the tumor develops can only be answered after further investigations.

Another speculation concerns the etiological factors which may bring about a condition in which the individual does not react after the injection of homologous protein. The internal secretions are to be seriously considered as etiological factors in producing this biological change.

CONCLUSIONS

A transient hyperglycemia occurs in rats after injection of adrenalin. The type of this hyperglycemia is not distinctive nor characteristic after inoculation with transplantable tumor, irrespective as to whether or not the tumor graft grows.

Heterologous protein extracts induce hyperglycemia in normal animals and in those bearing transplantable tumors, as well as spontaneous tumor-bearing animals.

Autologous protein extracts do not cause a hyperglycemia in normal or spontaneous tumor-bearing animals; they occasionally cause a hyperglycemia in animals bearing transplantable tumors, if the autologous tissue be the tumor itself.

Homologous protein extracts cause a hyperglycemia in normal animals and in animals bearing transplantable malignant tumors; they do not cause a hyperglycemia in animals in which spontaneous malignant tumors are growing.

Homologous protein, which is able to induce immunity against subsequent inoculation of a tumor, gives rise to a hyperglycemia, while autologous protein extract, which has not the power of conferring immunity, does not give rise to a hyperglycemia when injected into normal animals. A negative glycemic reaction, therefore, might be interpreted as an inability of the organism to produce antibodies against the substance injected. That such an interpretation is probably correct is shown by the fact that of animals bearing transplanted tumors, 75 per cent of those in which the tumor grows fail to give a glycemic reaction when injected with extracts of protein of the tumor, while those animals in which the tumor recedes give a glycemic reaction in 69 per cent of the cases. Animals in which the tumor recedes probably produce antibodies against the tumor, even though these cannot be demonstrated by any method at present known.

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SKIN INVOLVEMENT IN BREAST CANCER WITH REFERENCE TO ITS BEARING ON THE INTERPRETATION OF APPEARANCES OF TRANSITION BETWEEN NORMAL EPITHELIUM AND CANCER[1]

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Whether carcinoma ever extends by the transformation of normal epithelial cells into cancer cells at the advancing tumor margin has been a much debated question. The principal evidence in favor of spread in this manner is found in the histological pictures showing all stages of apparent gradual transformation of normal epithelial cells into malignant cells, seen notably in epitheliomata and in cancers of the intestine and duct cancers of the breast. If these are true pictures, their bearing upon the nature of cancer is of the first importance, indicating that the properties of malignancy may be conferred upon normal cells by an influence outside themselves, and that tumors need not necessarily grow exclusively from their own cells.

Borrmann (1), writing of epithelioma and arguing for the origin of tumors from foci of misplaced embryonic tissue, considers that the apparent transitions are to be explained as secondary union of the cancer with the normal epithelium. Ribbert (2), in a discussion of Paget's disease of the nipple, has expressed the same opinion: "Cancer here (of the breast) as elsewhere and like all other tumors, after it is once fully established, grows only from itself. This is a fundamental consideration. . . ."

Janeway (3) has summarized and endorsed the opposite view, that the pictures observed are true transformations. Writing of epithelioma, he says, "The new growth increases in size by a transforming influence upon the adjacent healthy epithelial cells with which it is in direct connection." More recently Ewing (4) has definitely expressed this view. In the breast, for instance, "some acinar and many duct carcinomas arising at one focus, gradually extend over adjoining areas by a gradual transformation of duct and acinar epithelium into neoplastic cells." A similar conception of the spread of carcinoma of the large intestine is mentioned (5). The question has not, therefore, been answered with finality and any evidence bearing on it is worthy of record.

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[1] This work was done during the tenure of a fellowship of the China Medical Board, Rockefeller Foundation.

The researches above referred to have been made by histological study of the relation of tumors to surrounding normal epithelium in which they have arisen. The tumor cells, especially in epitheliomata, still bear a more or less close resemblance to the surrounding normal cells and are of necessity in contact with them (unless the normal epithelium has been destroyed well in advance of the cancer), so that every opportunity is offered for the production of deceptive appearances of transition. It is reasonable to expect some light to be thrown on the question by a study of contact between malignant and non-malignant epithelia of different histological type. The problem has been approached experimentally from this point of view by Rous (6) who demonstrated that a transplantable adenocarcinoma (the Flexner-Jobling tumor) would unite directly with normal regenerating skin epithelium of a granulating wound to produce histological pictures of simple union and of *apparent* gradual transition, without evidence which could be interpreted as showing that the skin cells were actually changed into tumor cells. Previous work with simultaneous grafting of embryo tissues and tumor (7) had shown that the cells of an adenocarcinoma may unite secondarily with normal epithelial cells of quite different histological type and intermingle with them.

We are approaching the problem on experimental animals from a somewhat different point of view. But in the meantime it has seemed worth while to study the experiment, carried out by nature in the human subject, of allowing cancer to grow into contact with normal epithelium, which may be observed whenever cancer of the breast involves the overlying skin. The conditions are ideal, inasmuch as the tumor is one arising from the individual's own cells rather than from transplanted material, and, furthermore, the mammary epithelium from which the tumor arises is intimately related embryologically with the skin epithelium, while it is yet sufficiently different in histological appearance to be readily differentiated.

The object of this paper, therefore, is to report a study of the histological pictures seen when skin epithelium has been "exposed" to approaching mammary carcinoma and to show that in the cases studied, no pictures of transformation occurred, but that appearances of histological union were seen which, except for the well-marked difference in morphology between the cells of the two types of epithelium, might be mistaken for true transformations.

The processes which go on when deep carcinoma of the breast approaches the skin have been summarized by Ribbert (2): "When a cancer approaches the epidermis either it destroys it as a whole by compression, or its alveoli press on the basal layer, blend with it and break through it here and there, or they grow inside the epidermis, building in it epithelial nests and columns." Each of these processes has been observed in the material studied for this paper. The variation in the general relation of advancing carcinoma to overlying skin has been striking. In some instances a single column of cancer cells has approached and penetrated the entire thickness of the epidermis well in advance of the main tumor mass (fig. 1). In a number of cases, especially at the margins of ulcers, islands of cancer cells have been found immediately against the skin epithelium without the interposition of basement membrane. The arrangement of the groups of cancer and of epithelial cells, however, has not been such as to suggest a true union but only a very close apposition (fig. 2).

In another type of approach a large area of cancer has been circumscribed against the epidermis with a narrow margin of connective tissue intervening (fig. 3). In still other cases, extraordinary pictures of hypertrophy of the epithelium downward in fine processes interlacing with equally fine strands of cancer have presented themselves (fig. 4).

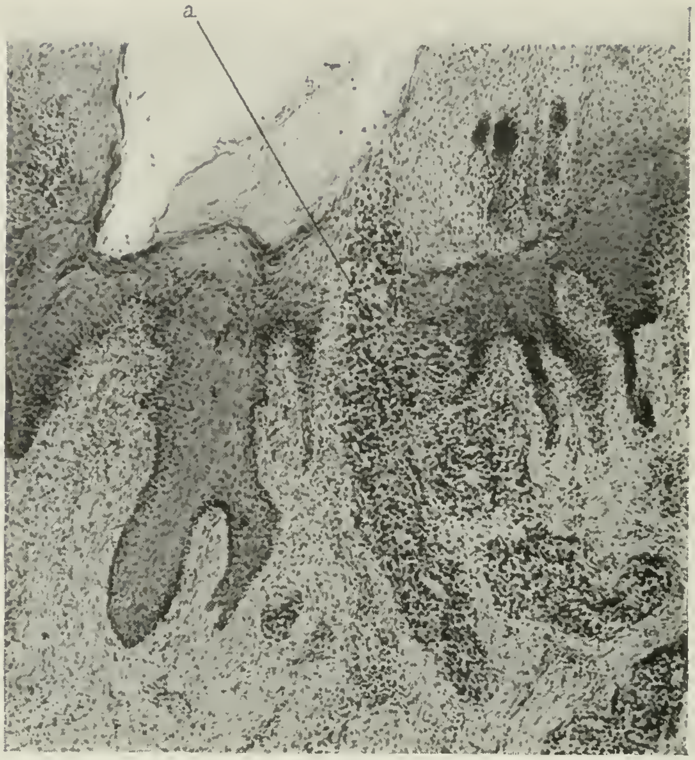

Fig. 1. Path. No. 23931

a, A single column of cancer cells penetrating the epidermis

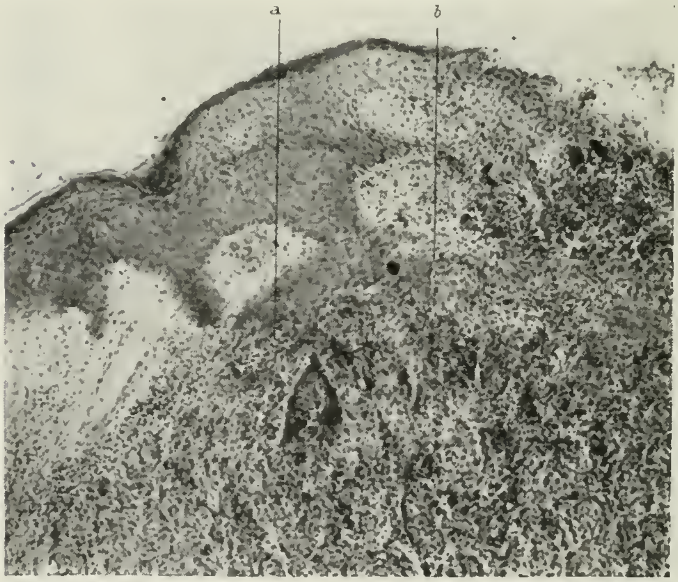

Fig. 2. Path. No. 25111

At an ulcer margin. *a*, An island of cancer cells in close apposition to skin epithelium which surrounds it on three sides. No basement membrane is seen, but there is no interdigitation of the two types of cells, the arrangement indicating only close apposition and not secondary union. *b*, Point of contact of another island of cancer with skin epithelium; basement membrane not yet destroyed.

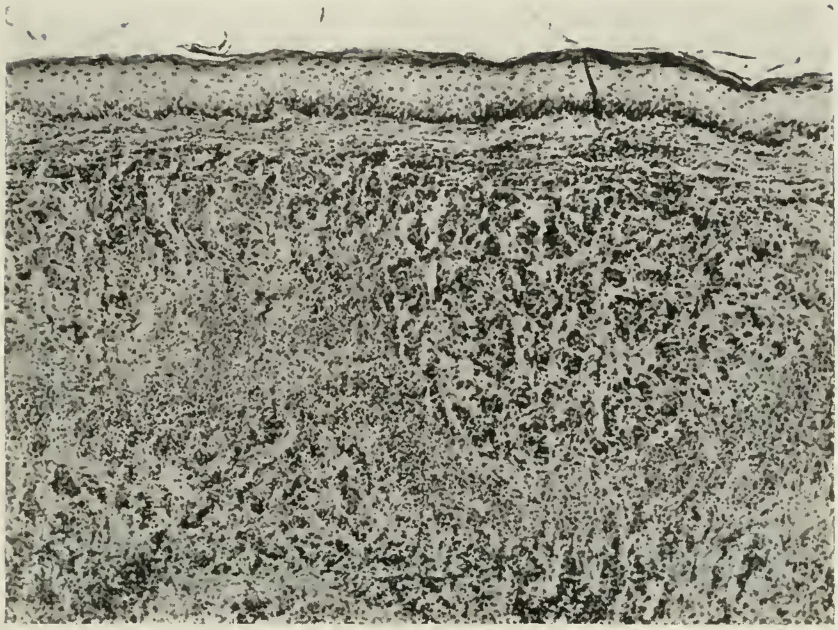

Fig. 3. Path. No. 23730

A second type of approach of carcinoma to skin. Broad area of cancer circumscribed against flattened epithelium with narrow zone of intervening connective tissue. No hyperplasia of skin epithelium. No secondary union.

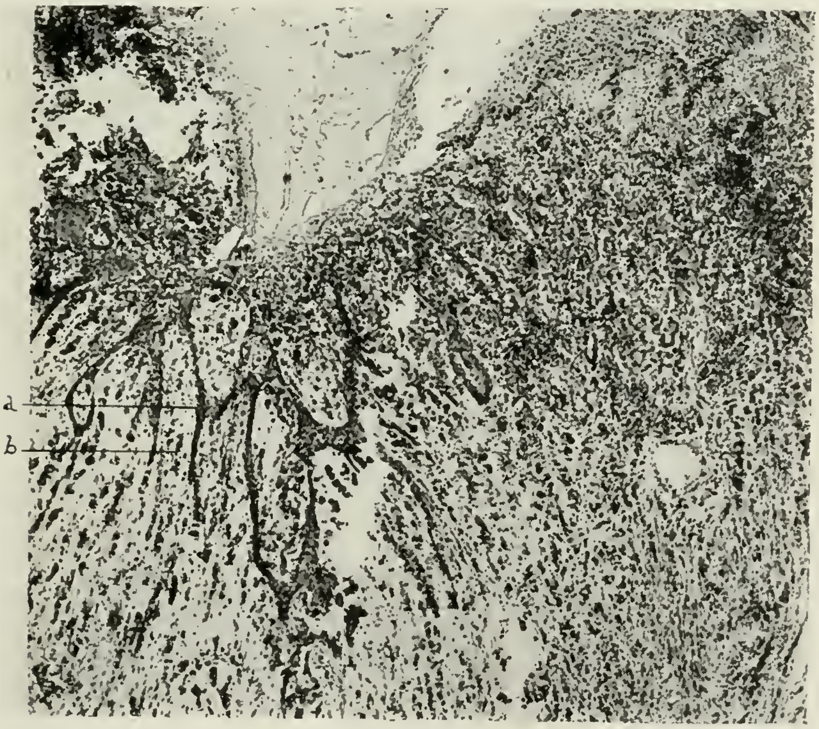

Fig. 4. Path. No. 14251

Third type of approach. Marked hyperplasia of skin epithelium in thin processes interlacing with fine strands of cancer. *a*, Process of skin epithelium. *b*, Strand of cancer.

It is in the last-mentioned type of picture that the main interest of the study has centered, for in the relation of the fine strands of cancer and the fine hypertrophic processes of skin epithelium have been found the appearances of secondary union, the interpretation of which is in question. Figure 5 shows a process of downgrowing skin epithelium which has preserved its basement membrane against the cancer strands at its side, but shows apparent union with the cancer cells at its tip. The connection between the two, evident under the microscope, is shown but faintly in the photomicrograph, and for this reason the picture is perhaps not as convincing as that shown in figure 6, from another part of the same specimen, in which a strand of cancer cells lies parallel to, and in intimate contact with, a process of epithelium and the highest cancer cell has definitely interdigitated with the skin cells. The change in its shape is obviously due to

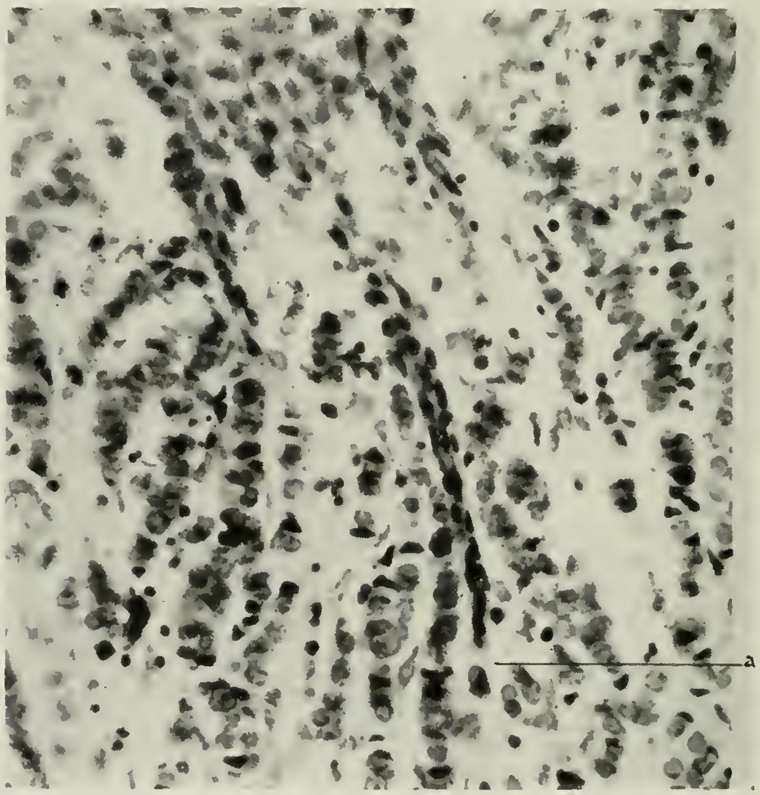

FIG. 5. PATH. No. 14251

a, Cancer cells in apparent secondary union with a narrow process of epithelium at its tip.

this interdigitation, and it cannot be regarded as a transitional form between normal and cancer cells. The non-malignant skin epithelium is readily distinguished by its fusiform or oval, small, deep-staining nuclei, its spindle-shaped cells with rather dark-staining protoplasm, arranged in a more or less orderly manner in the processes; the cancer cells by their large, irregular, more vesicular nuclei, their scant, ill-defined, and lightly-stained protoplasm, and the irregularity of their arrangement in strands and small islets. There are no intermediate types to be seen. If, however, the carcinoma were of the prickle-cell variety, confusion might easily arise in attempting to designate the last malignant and the first non-malignant cell.

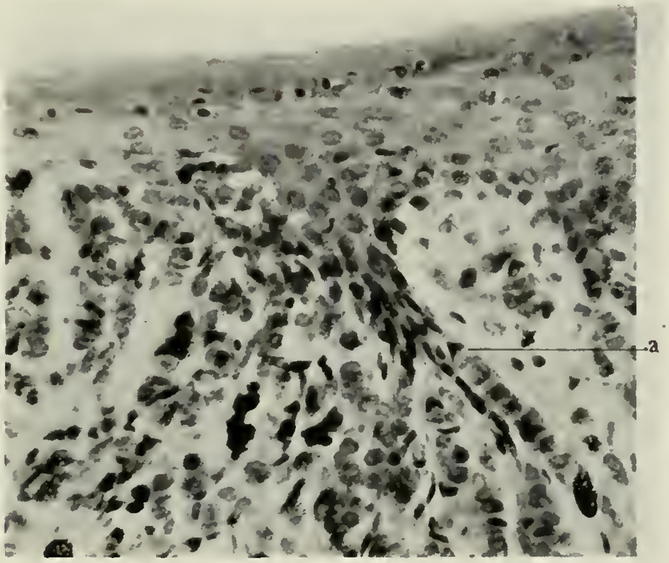

Fig. 6. Path. No. 14251

a, A cell at the end of a cancer strand interdigitating in apparent secondary union with cells of an epithelial process.

In figure 7 a cell belonging to a small island of cancer has interdigitated with the cells of a process of skin epithelium, approaching in this instance from the side. In spite of the apparent actual histological union it is possible, on account of the marked difference in morphology between the two types of cells, to designate with certainty the last mammary cancer cell and the first epidermal cells, and again no intermediate forms are to be seen.

The material on which this study has been made consists of 475 specimens of cancer of the breast of fully developed scirrhous or medullary type which have been received in the Surgical Pathology Laboratory of the Johns Hopkins Hospital during the past ten years. Of this number 74 showed histological involvement, either of the nipple or of the skin overlying the breast. In 24 of these, however, the cancer had reached only to the lower layers of the derma and showed close approach only to the hair follicles or skin glands—incidentally without showing any appearances of histological union with these structures. It has been considered, therefore, that true "exposure" of the epidermal epithelium to carcinoma has occurred 50 times in the 475 breast cancers observed. Of these 50 exposures 16 showed intimate approximation of cancer to skin cells, but in only 7 of these were seen pictures of apparent histological union such as is illustrated

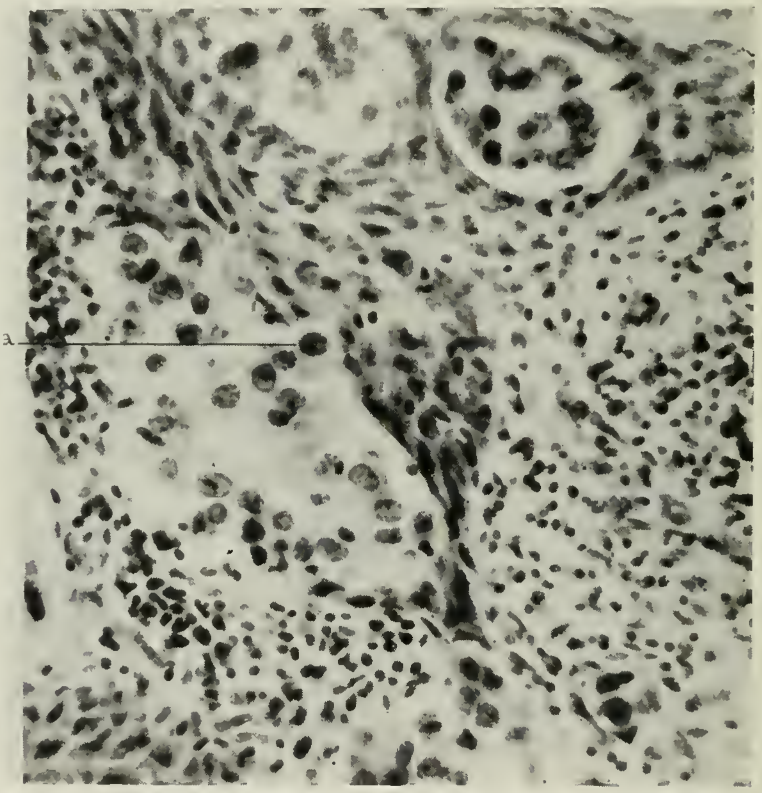

Fig. 7. Path. No. 12215

a, A cell from a cancer island interdigitating with cells of a process of skin epithelium. Approach from the side of the process.

in figures 5, 6, and 7. In none of these 7 cases of secondary union was seen any suggestion of transformation of non-malignant into malignant cells. Study of serial sections in a number of specimens has not changed the interpretation of the appearances at any given point.

It is of interest that all the pictures of apparent secondary union found in these cases occurred only where the skin epithelium was definitely hyperplastic, i.e., in those cases in which the skin over the cancer showed the type of reaction illustrated in figure 4. In the experimental work mentioned above, Rous also obtained secondary union between tumor cells and epithelium in hyperplastic activity, either regenerating epithelium on a granulating wound (6) or transplanted embryonic epithelium (7). Active growth of both of two tissues is therefore favorable, if not essential, for secondary union. If the uniting epithelia are dissimilar the cells of each retain their identifying characteristics.

These considerations suggest a reasonable explanation of the apparently perfect transition pictures seen between cancer and surrounding normal epithelium. Certainly hyperplasia of non-malignant epithelium is commonly seen at the margins of cancer, the effect of which is to alter the general morphology of the normal cells in many respects toward that of the tumor cells.[2] Cancer cells, on the other hand, vary from marked distortion to forms nearly, if not quite, normal in morphology. Gradual transition may easily be simulated by this variation of each type toward the other, the line of demarcation between cancer and normal epithelium being obscured by their secondary union.

SUMMARY

It is not the purpose of this paper to assert that cancer never extends by transformation at the periphery of normal epithelial into malignant cells, but an attempt has been made to show that, in a considerable number of cases in which conditions for

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[2] This reaction of normal epithelium is by no means specific for cancer. Exactly similar hyperplasia is characteristically observed in epithelium at the margins of benign epitheliomata and of ulcers due to tuberculosis, syphilis, or chronic inflammation. Vide, Councilman: Bull. Johns Hopkins Hosp. 1890, No. 2.

the occurrence of this transformation were theoretically very favorable, it has not been observed. Conclusions may be summarized as follows:

1. In 50 instances of "exposure" of epidermal epithelium to cancer approaching from below seen in 475 mammary carcinomas, no picture was observed which could be interpreted as transformation of normal into malignant cells.

2. In 7 cases, however, secondary histological union apparently occurred, which, but for the distinctive morphological appearance of the cancer and the skin epithelial cells, might be mistaken for transformation.

3. In so far as this evidence weighs, it is against the transformation of normal epithelial cells into cancer at the advancing margin of tumor.

4. The pictures of secondary union observed in these cases have occurred only in the presence of hyperplasia of the normal epithelium, and with this in mind a reasonable explanation is suggested of the apparent transitions seen at the margins of tumors, the cells of which are more nearly like those of the surrounding epithelium.

I am indebted to Dr. Joseph C. Bloodgood for permission to use the material in this laboratory and for criticism and suggestions; and to Mr. Herman Schapiro for the accompanying photomicrographs.

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THE RESPONSE OF THE ANIMAL ORGANISM TO REPEATED INJECTIONS OF AN ACTIVE DEPOSIT OF RADIUM EMANATION

INTRAVENOUS INJECTIONS IN DOGS

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Following intravenous or subcutaneous injections of an "active deposit" of radium emanation, the writer found (1) in the case of white rats, that pathological changes had resulted in the liver, kidneys, spleen, bone marrow, etc., of these animals; and since the methods employed in these experiments were being used in the treatment of certain types of human cancer, it was decided to run a parallel set of experiments on a larger animal, the dog, and therein to determine the severity of the physiological reactions. To this end a complete urine analysis was made each day, before and after the treatments; frequent blood counts and differentials were made; the temperature and weight reactions were recorded, and finally a histological study was made of the organs removed at autopsy.

This experiment was undertaken in collaboration with Miss Ruth Theis of the chemical department of the Memorial Hospital. We will report elsewhere on the detailed findings of the urine analysis, and mention of them will be made in this paper only in so far as they aid in the interpretation of what might be called the general clinical aspects of the problem (2).

APPARATUS AND METHODS

Two female dogs were the subjects of the experiments. Dog I was a Dalmatian and weighed $32\frac{1}{2}$ pounds when first obtained

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by the laboratory. Dog II was a mongrel, with the bull dog type predominating, and weighed nearly 28 pounds at the beginning of the experiment.

While the dog was being treated it was catheterized at a definite time each day. It was kept in a metal metabolism cage, in which the urine and feces were collected separately. The catheterized urine completed the twenty-four hour specimen that was used as a basis of comparison of the urine analysis. Although the dogs were under observation for a comparatively long time, eighty days in the case of dog I, no bladder infections were encountered. Each dog was trained to lie quietly on its back in an ordinary trough shaped animal board, its legs were loosely tied, and catheterization was effected by means of a silver catheter. These arrangements made it possible for one person to do the work unaided by an assistant. The bladder was emptied and then washed two or three times with sterile warm water, which was added to the day's collection of urine, together with the washings from the floor of the metabolism cage. After the washing with sterile water, the bladder was again flushed with a saturated solution of boric acid. Immediately after this operation the dog was fed its daily amount of food previously prepared and measured according to a definite formula.

The "active deposit" of radium emanation was prepared as described in the previous article (1). It consisted of a solution in which radium emanation had been previously deposited upon common salt, which subsequently was dissolved in sufficient sterile water to bring the liquid to the strength of a physiological salt solution. This solution contained all the properties of radium metal itself.

The intravenous injections were effected as follows: First the dog was placed on its back on the animal board, and held firmly in position. Then its ear, previously shaved, was warmed by several applications of hot cloths until the ear veins became prominent. The ear was washed with alcohol, and by means of a fine needle and a 2 cc. Luer syringe, the activated solution was slowly allowed to enter the general circulation. About 2 cc. of solution were injected at each treatment. The syringe was covered by a lead shield which acted as a protection for the fingers of the operator.

The blood samples were also obtained from the ear. Rectal temperatures were taken each day, and at more frequent intervals during the period of treatment. The dog was weighed every day during the time when significant weight changes were occurring.

Throughout the experiment an injection of the active deposit was not repeated until the examination of the urine showed that the metabolism of the animal had recovered from the previous treatment.

A. EXPERIMENTAL RESULTS FOR DOG I

First treatment. In the case of dog I the treatments were started at the beginning of March and continued until the last week in the following May. During this period the animal received four intravenous injections of the radioactive solution, the total amount being 231.8 mc. The first treatment consisted of an injection of 95.3 mc. This was followed by a period during which the animal was inactive. The feces were semifluid and food was eaten only after much persuasion. This condition lasted for only two days, for on the morning of the third, the dog's appetite had completely recovered, and the feces were again normal. The second treatment was not given until a month later, and during that time the animal appeared normal, except for a temporary inexplicable loss of appetite on the thirteenth day of the experiment, and on the twenty-third and twenty-fourth days.

Figure 1 shows that following the first treatment there was a considerable drop in the number of white blood-cells, which reached their lowest point on the eleventh day of the experiment. The number of cells before treatment was 10,250 and ten days later their total was only 4200, but from then on there was a steady recovery until the normal number was again reached just before the second treatment. Immediately after the injection, the number of red blood-cells remained practically unaltered, and, in fact, while the white cells were rapidly decreasing the reds held their own and slightly increased in number.

As indicated in table 1, on the eleventh day of the experiment the differential showed that an interesting change had taken place in the composition of the white blood-cells. The percentage of polynuclear leucocytes had gradually been reduced from 83 to 64, and the relative percentage of lymphocytes correspondingly increased from 16 to 29, while the eosinophiles went from 1 to 6 per cent, and 1 per cent of myelocytes was found. There was also a slight decrease in hemoglobin. Before the next treatment the differential was again normal.

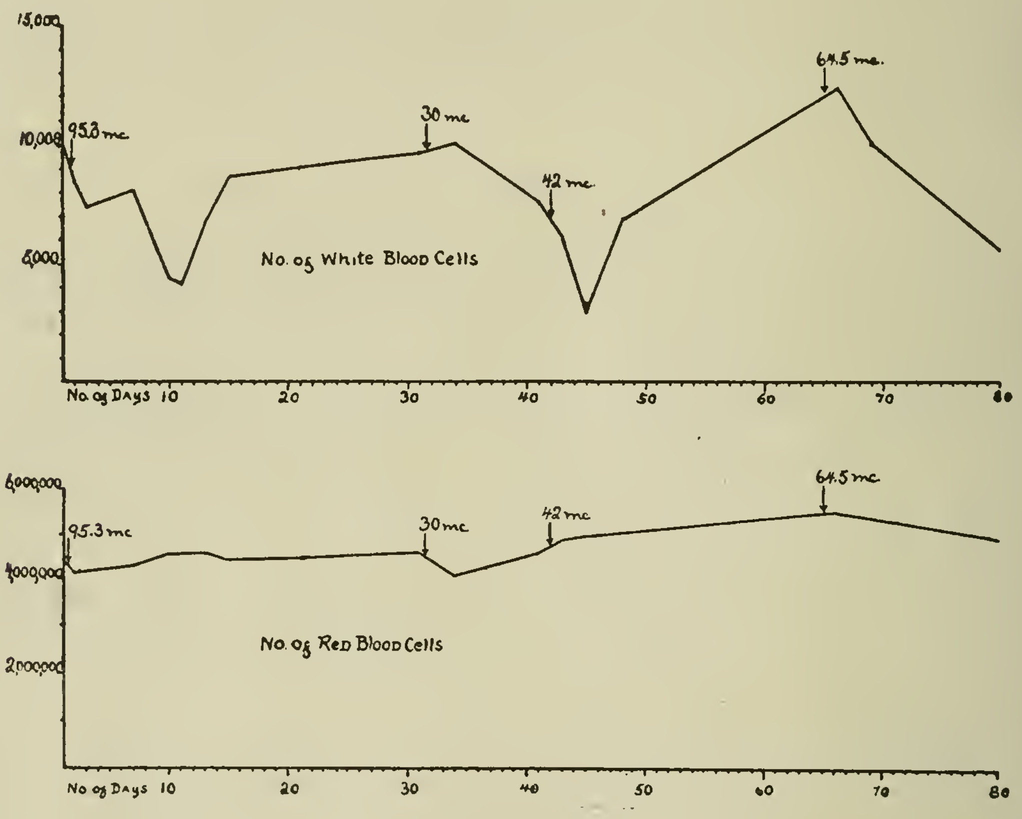

Fig. 1. Record of Red and White Blood-Cell Changes in Dog I Following Repeated Intravenous Injections of the Active Deposit of Radium Emanation

The arrows indicate the time when the injections were given, and immediately above them is indicated the dosage, expressed in millicuries.

The examination of the urine showed that in response to the injection there was an increase in the total nitrogen and urea, and a considerable increase in the uric acid and phosphates, but, as in the case of the blood changes, the metabolism was also back to normal before the next treatment.

Second treatment. The second injection occurred on the thirty-first day of the experiment. A much smaller dose was given, consisting of 30 mc., and save for the fact that the feces were slightly more fluid than normal, the dog remained well and active. As shown in figure 2, the temperature during this period showed considerable irregularity, with generally low temperatures for a few days.

TABLE 1

Complete record of the blood counts for dog I

RADIUM TREATMENTS	NUMBER OF DAYS	NUMBER OF RED CELLS	NUMBER OF WHITE CELLS	PERCENTAGE HAEMOGLOBIN	PERCENTAGE POLYNUCLEAR LEUCOCYTES	PERCENTAGE LYMPHOCYTES	PERCENTAGE EOSINOPHILES	PERCENTAGE MYELOCYTES
	1	4,400,000	10,250	80	83	16	1	0
First radium treatment, 95.3 mc.	1							
	2	4,112,000	8,300	80				
	3	4,256,000	7,450	70	82	18	0	0
	7	4,336,000	8,200	85				
	10	4,600,000	4,400	85	70	28	2	0
	11		4,200	75	64	29	6	1
	13	4,640,000	6,900	75	77	20	3	0
	15	4,440,000	8,900	75	85	11	4	0
	22	4,500,000	9,300	75				
	31	4,664,000	9,900	80	83	15	2	0
Second radium treatment, 30 mc.	31							
	34	4,136,000	10,200	80	86	14	0	0
	41	4,690,000	7,600	80				
Third radium treatment, 42 mc.	42							
	43	4,890,000	6,150	80				
	45	4,976,000	2,900	80				
	48		6,950					
Fourth radium treatment, 64.5 mc.	65							
	66	5,544,000	12,500	75	81	17	2	0
	69	5,400,000	10,100	80	76	17	7	0
	80	4,980,000	5,600	80	75	25	0	0

Three days after the treatment the white blood-cells were slightly increased in number, but from that point until the third treatment the cells were decreased about 25 per cent. The red blood-cells were slightly lowered, but regained their normal number before the next treatment. No significant change was noted in the differential.

There was a slight rise in the total nitrogen, the urea, and the total phosphates of the urine, while the uric acid remained at about the same level.

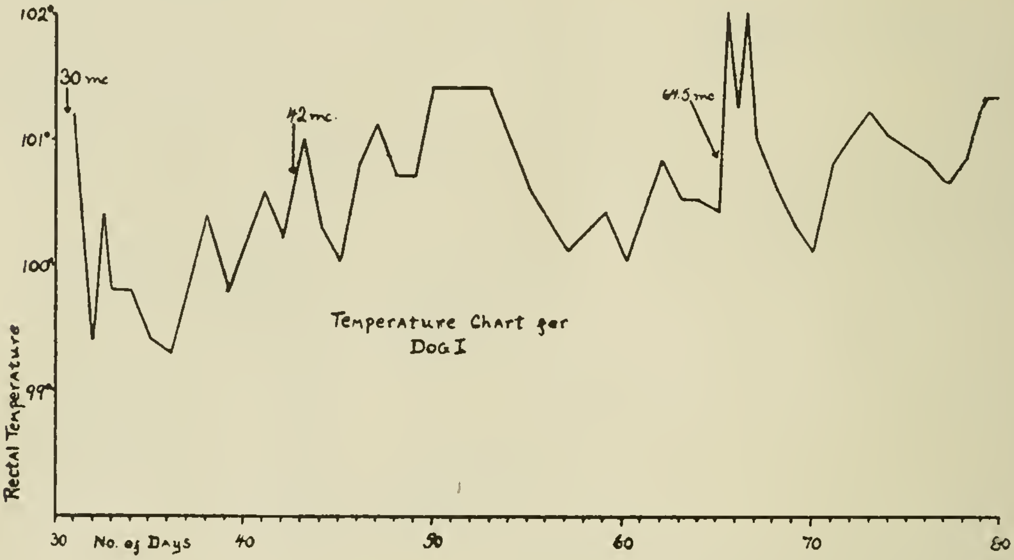

FIG. 2. TEMPERATURE CHART FOR DOG I

The arrows indicate the time when the injections were given. The record was not begun until after the second treatment.

Third treatment. A third injection of 42 mc. was given on the forty-second day of the experiment. It was followed by a slight amount of diarrhea, loss of appetite which lasted for ten days, and a decrease in weight amounting to two pounds. There was also a sharp rise in temperature, with irregular and generally high temperatures for several days, during which the feces were hard and dry. There was a further reduction in the number of white blood-cells to 2900, which number was recorded three days after the injection. From this point the white cells rapidly increased, until at the time of the next treatment they were 12,500 in number.

The hemoglobin remained at 80 per cent. The number of red blood-cells increased, so that before the animal was again injected there were approximately a million more red cells present than there were at the beginning of the experiment. Just after the third treatment it was noted that the blood tended to clot with unusual rapidity.

The examination of the urine showed a considerable increase in the total nitrogen, urea, creatinine, uric acid, and the total phosphates.

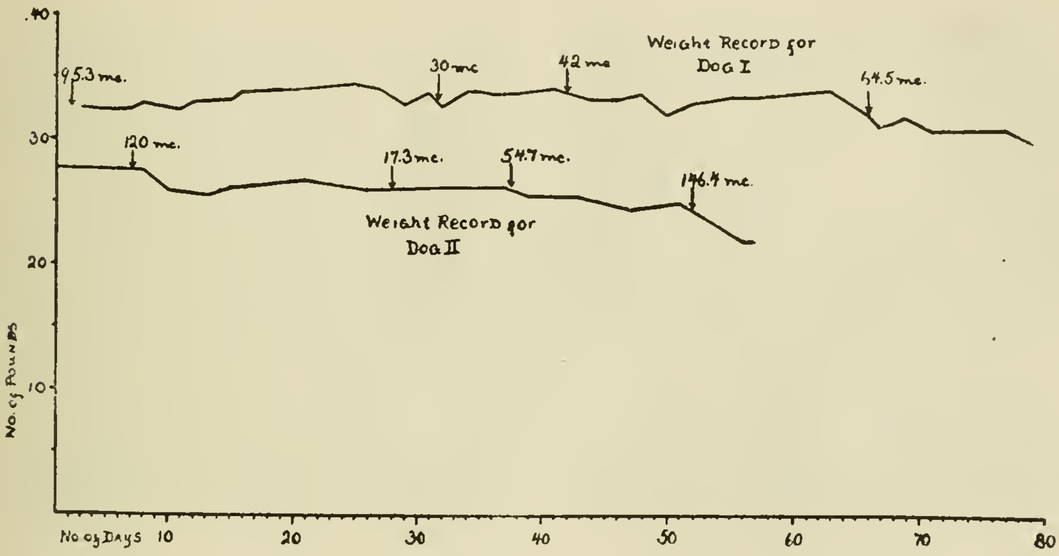

Fig. 3. Record of Weight Changes for Dog I and Dog II Following Repeated Intravenous Injections of the Active Deposit of Radium Emanation

The arrows indicate the time when the injections were given

Fourth treatment. Sixty-four and a half millicuries were injected on the sixty-fifth day of the experiment. This treatment was followed by severe vomiting, constipation, the excretion of a small amount of solid, dry feces, and a sudden rise in temperature to 102°. At the end of the following day the temperature again reached the same high level before finally receding. The animal refused to eat for two days, and as indicated in figure 3, there was a steady loss of weight amounting to $3\frac{1}{4}$ pounds. The animal was inactive and obviously seriously ill.

The white blood-cells were reduced in number to 5600, but the red blood-cells were only slightly decreased. The differential showed a relative increase in the number of lymphocytes at the expense of the polynuclear leucocytes. The fact that the animal refused to eat after the fourth treatment made the chemical analysis of the urine unreliable. There was, however, a decided increase in the amount of urine excreted each day.

At this time the dog was killed by means of ether.

Histological report for dog I

The histological study of the organs showed an intensely congested liver, with some capillary varicosities and severe granular degeneration of most of the parenchyma cells. The kidneys were also intensely congested, showing a moderate amount of degeneration of the tubule cells. The malpighian bodies of the spleen were prominent; the organ was congested, and the pulp considerably drained of cells. There was marked congestion of the colon associated with active mucus production. The lymph-nodes showed extreme congestion, especially of the pulp. The lymph-follicles were reduced in size, but the reticulum cells were generally increased in number. No definite changes were noted in the lungs, stomach, small intestine, or thyroid.

B. EXPERIMENTAL RESULTS FOR DOG II

First treatment. The experimental work with dog II began in the first part of June and continued until the end of July. Four injections were given, the total amount of radium emanation amounting to 338.4 mc. The initial treatment consisted of 120 mc. injected on the seventh day of the experiment. As indicated in figure 4, this was followed by irregularly lowered temperature for several days following the injection. No gastric symptoms were noted until three days after treatment, when vomiting occurred; the animal became dull and inactive, and refused most of its food. Figure 3 shows there was a loss in weight amounting to 2 pounds, occurring within six days. As shown in figure 5 there was a marked decrease in the number of white blood-cells;

from 14,400 before treatment to 2150 seven days later. There was a temporary fall in the hemoglobin from 85 to 75 per cent, accompanying a decrease of about two million red cells. As shown in table 2, the differential remained normal, except for a slight relative increase of lymphocytes. The examination of the urine showed a large increase in uric acid and the total phosphates.

Second treatment. A small dose, consisting of 17.3 mc., was injected on the twenty-eighth day of the experiment. After this treatment there was at first a temporary drop in the body temperature, followed by a rapid increase at the end of the following day, at which time 102.5° was reached. No other change was noted in the animal's general condition. It remained active, and ate with its usual good appetite. There was a steady decrease in the number of white blood-cells, the hemoglobin remained normal, but the red blood-cells continued to increase slowly. The weight remained stationary, as indicated in figure 3. The analysis of the urine showed only a slight increase in the total nitrogen, the creatinine, the uric acid, and the total phosphates.

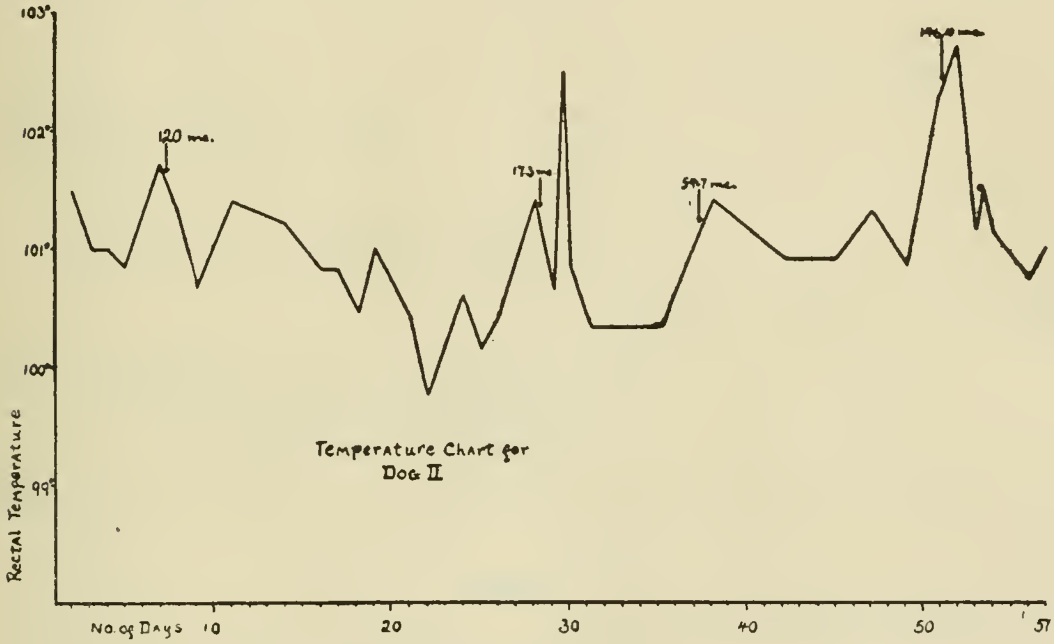

FIG. 4. TEMPERATURE CHART FOR DOG II

The arrows indicate the time when the injections were given

Third treatment. On the thirty-seventh day of the experiment the animal received an injection of 54.7 mc. Following this there was a slight temporary increase in temperature, during which time the animal was somewhat inactive for three successive days, but showed no signs of digestive disturbances, except for a temporary refusal of food on the fourth day. During this period the animal lost 1½ pounds in weight. The number of red and white blood-cells remained fairly constant, and yet the differential showed a relative decrease in the percentage of lymphocytes, with a corresponding relative increase in the polynuclear leucocytes and the formation of a few eosinophilic cells. The urine showed an increase in the total nitrogen and the phosphates, while the creatinine stayed at about the same level.

TABLE 2

Complete record of the blood counts for dog II

RADIUM TREATMENTS	NUMBER OF DAYS	NUMBER OF RED CELLS	NUMBER OF WHITE CELLS	PERCENTAGE HAEMOGLOBIN	PERCENTAGE POLYNUCLEAR LEUCOCYTES	PERCENTAGE LYMPHOCYTES	PERCENTAGE EOSINOPHILES	PERCENTAGE MYELOCYTES	PERCENTAGE NORMOBLASTS
	3	6,400,000	13,800	85	79	19	1	0	1
	4	5,924,000	14,400	85					
First radium treatment, 120 mc.	7								
	8	5,600,000	8,900	85	82	17	1	0	0
	9	5,280,000	4,550	80	79	18	3	0	0
	11	4,880,000	2,815	75	84	16	0	0	0
	14	4,680,000	2,150	80					
	28	4,976,000	5,700	85	75	23	2	0	0
Second radium treatment, 17.3 mc.	28								
	29	5,250,000	4,450	85					
Third radium treatment, 54.7 mc.	37								
	38	5,800,000	3,650	85	83	14	3	0	0
	49	5,814,000	4,100	80	85	12	3	0	0
Fourth radium treatment, 146.4 mc.	52								
	53	5,200,000	2,600	85	95	3	2	0	0
	57	4,520,000	1,400	85					

Fourth treatment. The final injection was a large one, and consisted of 146.4 mc. administered on the fifty-second day of the experiment. Just before the treatment the temperature went as high as 102.3°, and after the treatment it reached 102.7° and then as suddenly receded. A few hours after the treatment a considerable amount of mucus covered vomitus was found in the cage, as well as a large quantity of fecal matter, semi-solid and odorless, and also covered with mucus. The animal refused to eat during the rest of the experiment except for an occa-

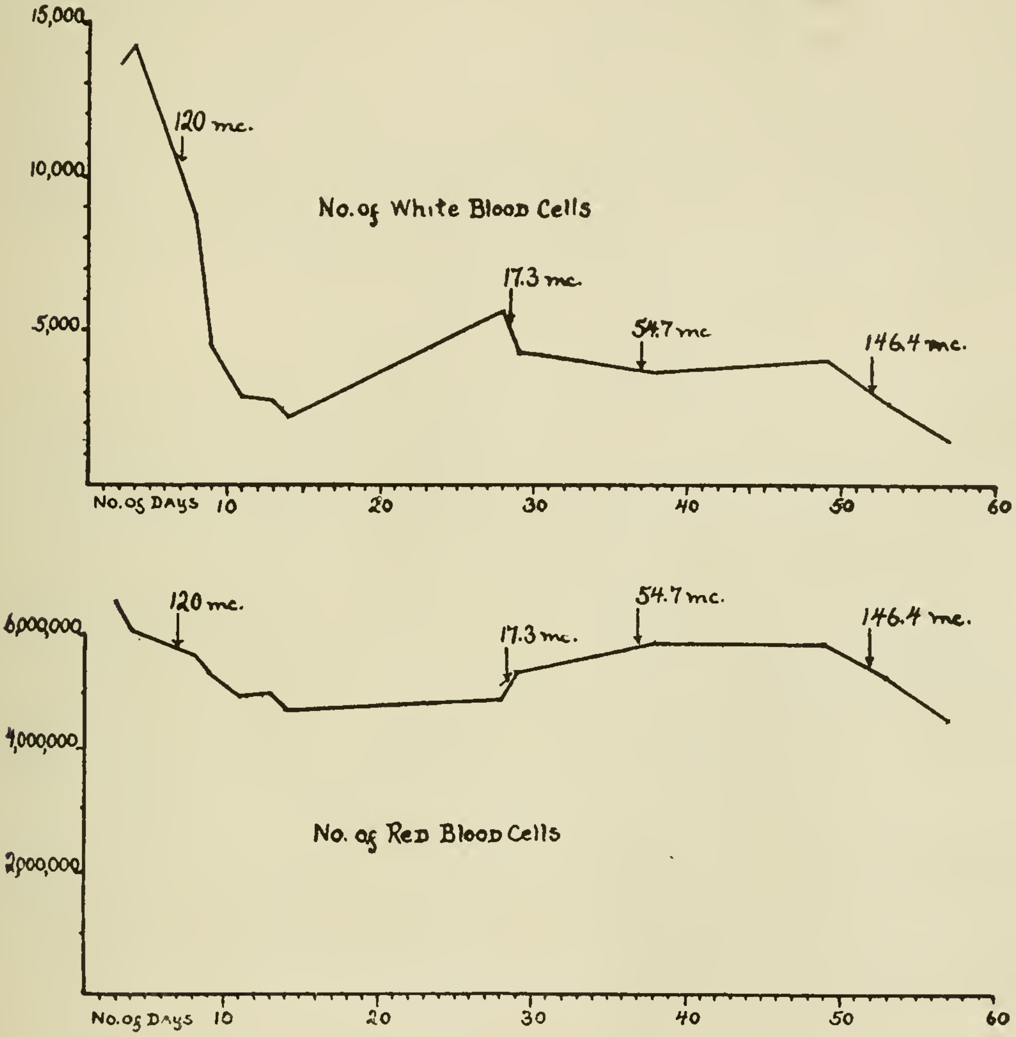

Fig. 5. Record of Red and White Blood-Cell Changes in Dog II Following Repeated Intravenous Injections of the Active Deposit of Radium Emanation

The arrows indicate the time when the injections were given sional small amount of food. It lost 2 pounds in weight within five days, and remained seriously ill and inactive. The white blood-cells decreased still further in number until at the end of the experiment they were as low as 1400 cells. The differential showed a very decided reduction in the percentage of lymphocytes. These cells were reduced to 3 per cent, while a corresponding relative increase in the polynuclear leucocytes was recorded, amounting to 95 per cent. The hemoglobin remained normal and the red blood-cells were but slightly decreased in number. The urine analysis was unreliable, because the animal refused to eat. The animal was killed by ether anesthesia.

Histological report for dog II

The histological study of the organs showed a general fatty and granular degeneration of the liver, associated with capillary congestion. The kidneys showed a slight granular degeneration of the tubule cells, with general venous congestion. There was a marked old thickening of the trabeculae of the spleen, a heavy pigmentation of the parenchyma cells, associated with small and scanty follicles. The splenic pulp showed considerable congestion and fibrosis. The bone marrow from the head of the femur was devoid of lymphoid cells, which were largely replaced by fat. The lungs were congested, and showed a slight degree of emphysema. There was a small amount of catarrhal exfoliation of the sinus cells of the lymph-nodes. The pancreas, the thyroid and parathyroid, the stomach, and the small intestine showed no structural changes.

DISCUSSION AND SUMMARY OF RESULTS

The results of these experiments are interesting in that they show that very decided physiological reactions follow repeated intravenous injections of an active deposit of radium emanation. Clinical data have established the fact that external applications of radium or x-rays will produce decided changes in the composition and number of blood cells, and the results of this investigation show that the same changes may be obtained by injecting intravenously the active deposit of radium emanation.

In the case of external applications of filtered radium the physiological changes are produced mainly by gamma ray activity, while in the case of the radioactive solution used in the present experiments, the effect is largely due to alpha ray activity. The results show that following such intravenous injections there is a prompt reduction in the number of white blood-cells, which was especially well illustrated in the case of dog II, whose white cells were reduced from 14,400 before the treatments to 1400 at the end of the experiment. The terminal dose for the same animal also produced a remarkable reduction in the relative percentage of lymphocytes. As shown in table 2, after repeated doses of the radioactive solutions, the percentage of lymphocytes was reduced from 19 before treatment to 3 after the fourth injection. In this case, dog II, the four injections totaled 338.4 mc., and the last dose that preceded the sudden drop in lymphocytes, was a very large one, 146.4 mc. In both animals, however, after an initial moderate dose of the active deposit there was a gradual increase in the relative percentage of circulating lymphocytes in the blood. Dog I, that received 231.8 mc. in four injections (the total dose was 106.6 mc. less than the amount given to dog II), showed a terminal increase in the relative percentage of lymphocytes after moderate doses, which however were sufficient to reduce considerably the total white blood-cell count.

If it is true that following the instances where moderate doses of the active desposit of radium emanation were given to these animals there was an increase in, or a tendency to an increase in, the actual number of lymphocytes in the body, not merely those in the circulation, then these results may be considered similar to those of Murphy (3), of Murphy and Morton (4), and, more recently, of Murphy and Nakahara (5 and 6), in their experimental work concerning lymphoid destruction and the stimulation of the lymphoid elements in animals after exposure to *x*-rays. It is possible that such was the case, but the histological data at hand are not sufficient to settle the point.[1]

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[1] In order to determine the presence of lymphoid stimulation it will be necessary to kill the treated animal after a single moderate dose, and not to continue with larger doses as was done in these experiments. Further experiments are planned to elucidate this subject.

The present experiments show that the apparent red blood-cell destruction was slight in comparison with the white blood-cell changes. In the case of dog I, see figure 1, an initial injection of 95.3 mc. of radioactive solution was promptly followed by a constant increase in the number of red blood-cells, which remained above normal until the second injection was given. This increase, however, may not have exceeded the limits of variability normal to the changes in the number of red blood-cells. After the second treatment, there was a sudden return to the normal number, followed by gradual increases in the red blood-cells, which continued to remain above normal from then on until the end of the experiment. Dog II lost about 25 per cent of its red blood-cells after an initial dose of 120 mc. This was no doubt a significant response. This loss was followed by a gradual increase after the injection of the two succeeding moderate doses of 17.3 mc. and 54.7 mc. respectively. The large terminal dose of 146.4 mc. again reduced the number of red cells to their previously low figure.

Comment on the subject of these red blood-cell changes brings up a point similar to one discussed in regard to the possibility of the stimulation of lymphoid production following intravenous injections of the active deposit solutions. Again the histological data are not sufficient to show the presence of increased red blood-cell production in the bone marrow, etc. How much of the apparent increase in the circulating red blood-cells was due to the attending diarrhea, or other undetermined conditions, is still unknown.

During the period in which the white blood-cells were being materially reduced there was a decidedly noticeable tendency for the blood to clot with considerable rapidity. This condition may have been brought about by the possible liberation of enzymes set free as a result of the destruction of large numbers of blood-cells.

In a previous work of the writer in which white rats were used as subjects, it was found that following lethal injections of the active deposit of radium emanation, the animals died after showing symptoms of marked enteritis. This subject was discussed, and the phenomenon attributed to the fact that experimental data tend to show that a considerable amount of the radioactive substance was deposited in the intestinal tract. In the case of the dogs of the present investigation similar digestive disturbances were noted. After the first injection of 95.3 mc. dog I became inactive, diarrhea was present, and the dog refused to eat; but this condition lasted for only two days. The second injection was smaller, 30 mc., but following it the animal remained well and active, and showed no signs of digestive disturbances. The third injection consisted of 42 mc., and was followed by a slight amount of diarrhea, loss of appetite, and a reduction in weight. The fourth treatment, consisting of 64.5 mc., resulted in the animal refusing its food altogether, severe vomiting, and a further loss in weight.

The reaction of dog II to an initial dose of 120 mc. was interesting in that no digestive disturbances were noted until three days after the treatment, when vomiting occurred, and the animal became dull and inactive and would eat only a part of its food. The following injection consisted of 17.3 mc., and, as in the case of the small injection for dog I, it produced no digestive disturbances. Even with a dose of 54.7 mc., the third for dog II, no physical changes were noted, except that the animal was somewhat inactive for three days, and refused to eat on the fourth. The terminal large dose, however, 146.4 mc., produced a prompt reaction in the case of dog II. Vomiting was severe, a large amount of mucus covered feces was extruded, the animal refused to eat altogether, and lost two pounds in weight within five days.

Similar, but not as severe, intestinal disturbances were noted by Berg and Welker (7) in their work on the metabolism of dogs in nitrogenous equilibrium. They found, however, that no gross symptoms, except diarrhea, resulted from the administration by mouth of a preparation of 240 activity of radium bromid.

As shown in figure 2 and figure 4 the limits of the temperature changes indicate a considerable daily variation. It is thus difficult to make any definite statements concerning temperature reactions in response to this type of radium treatment. The sharp rise in temperature in response to the terminal dose that was given to dog I is no doubt beyond the limits of the probable error of the variability for that case, and indicates a definite attempt on the part of the organism to adjust itself to the toxic disturbances brought about by the radium treatment. This toxemia probably resulted from the relatively sudden destruction of a large amount of cellular material which had to be eliminated by excretory organs, already damaged by previous radium treatments and passing through processes of degeneration. A similar temperature reaction probably holds good in the case of the second treatment for dog II, although the dose in this case was a small one, and again in the terminal reaction for the same animal, although why the treatment should be immediately preceded by a high temperature as well as followed by one, is still unanswerable.

The effect of repeated injections of an active deposit of radium emanation upon the weight of the animal is clearly shown in the case of dog II, see figure 3. There was a gradual almost constant decrease, amounting to about 25 per cent of the original weight.

The metabolic changes showed in the main that the total nitrogen content of the urine, the urea, the creatinine, the uric acid, and the total phosphates were markedly increased, probably as a result of active tissue destruction, and also to some extent due to the interference of the proper functioning of the excretory organs, as a direct result of the injections of the active deposit.

That active degenerative changes had occurred as a result of the radioactive injections is shown by an examination of the microscopical sections of the organs. These results resemble those obtained by the writer after similar injections in white rats. In both experiments there was considerable congestion in the principal organs, but the more pronounced degenerative changes in the white rats were far more severe than those that occurred in the dogs.

As previously stated in detail, a considerable amount of degeneration was found in the liver and kidneys of the animals. The spleen was also considerably altered, while it is to be noted that the bone marrow from the head of the femur in dog II was devoid of lymphoid cells. The lymph-nodes as well as the lungs showed pathological changes in the case of dog II. The intestinal tract of dog II apparently showed no definite changes, while in the case of dog I, the only pathological changes that were noted were found in the colon, which was markedly congested, and showed signs of active mucus production. The stomach, thyroid, pancreas, and musculature presented no definite changes.

From a consideration of the results of the investigation as a whole, one may say that in the intravenous use of the solution form of the active deposit of radium emanation, as a therapeutic agent in cancer or other diseases, it is necessary to keep in mind the fact that after an initial dose of such radioactive substance the animal organism is irretrievably altered, and from then on will not give the same reaction to a repetition of the initial dose. The organism, as seen in the dogs of this experiment, is able to compensate for a severe initial dose of the radioactive substance, but after a certain point has been reached the natural protective adaptations on the part of the organs affected become inadequate to meet the demands of the organism as a whole, and the effects of the intoxication are greatly aggravated.

CONCLUSIONS

1. Large intravenous doses of an active deposit of radium emanation produce a considerable reduction in the number of white blood-cells, but while the white cells may be reduced by as much as 80 per cent of their total number, from the effects of an initial dose, the simultaneous reduction in the number of red blood-cells is less, and amounts to a reduction of about 25 per cent.

2. Repeated doses, amounting to a total of 338.4 mc. distributed in four intravenous injections, apparently produce a very marked decrease in the number of circulating lymphocytes of the blood.

3. Digestive disturbances, such as severe vomiting and diarrhea, followed large doses of the radioactive solutions, and were associated with a considerable reduction in the body weight.

4. In several cases, a rise in body temperature followed the treatments, suggesting an adaptive reaction on the part of the animal organism to meet the toxic condition produced by the relatively sudden destruction of a considerable amount of cellular material.

5. The metabolic changes, as determined by the daily urine analysis showed that the larger injections of the radioactive solutions were followed by very decided increases in the total nitrogen content of the urine, the urea, creatinine, uric acid, and the total phosphates.

6. Relatively small or moderate doses of the radioactive solution, if administered after the organism has already been injured by a previous injection, even when the metabolism is again back at normal, produce definite changes in the chemical content of the urine, indicative of destructive changes within the organism. These injections may or may not be followed by gross symptoms of digestive disturbance.

7. The histological study of the organs showed considerable congestion in the principal viscera. The liver showed a general fatty and granular degeneration, the kidneys showed a granular degeneration of the tubule cells, and the spleen presented considerable congestion, while the splenic pulp was largely drained of cells. The bone marrow was devoid of lymphoid tissue, which was largely replaced by fat. There was extreme congestion in the pulp of the lymph-nodes in one animal, associated with a reduction in size of the lymph-follicles. The lungs in one animal were normal, while in the other, which received the larger total of the radioactive solution, they were congested and showed slight signs of emphysema. The colon in one of the animals was markedly congested, and showed signs of active mucus production. The other organs showed no definite changes.

8. When the active deposit of radium emanation is used intravenously as a therapeutic agent, great care should be taken to grade the dose in accordance with the general physical condition of the patient, which should be determined by frequent urine and blood analyses; and if more than one dose is given during the treatment, the second dose should be made smaller in accordance with the strength of the original dose.

ACKNOWLEDGMENT

The writer wishes to express his indebtedness to Dr. James Ewing for his aid in the interpretation of the pathological results, and to Miss Ruth Theis and Dr. Stanley Benedict, who planned the chemical scope of the experiment.

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A STUDY OF THE OXIDASE REACTION WITH A-NAPHTHOL AND PARAPHENYLENDIAMINE IN TUMORS

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From time to time biological studies dealing with color reactions obtained in tissues and due to oxidation have been reported. These so-called oxidase reactions may be obtained by many different substances, belonging mostly to the phenols; among them are the two employed in this study. The development of color in the reagent depends primarily upon changes in its surface properties inducing oxidation, and these may be modified by contact with different tissues.

The various gradations of color indicate the amount of oxidation occurring, and since oxidation involves the acquisition of positive charge the tissue reaction is an index of the available positive charge which can be transferred by that tissue. In the following pages it is shown that the degree of color can be either increased or decreased within certain tissues. This we believe is closely related to potential of tissue. The less susceptible the tissue is to the reducing action of the reagent, the smaller the amount of color appearing and vice versa. If the conditions of the experiments are kept comparable variations of color in any one tissue, therefore, become a measure of the alterations in the potential energy of the tissue in question.

The early literature of the occurrence of oxidases in animal tissue deals almost entirely with the polymorphonuclear leucocytes and their precursors, the myelocytic cells and it is only in recent years that the study of the oxidase reaction has been extended to tissue other than white blood cells. It is not surprising, therefore, that the work reported on the oxidase reaction in tumors is not extensive. We have been able to find in the literature only three references to the occurrence of oxidase in tumor cells, and in two of these the tumors were derived from blood elements. Thus in 1917, Forman and Warren (1), accepting the synthesis of indophenol blue in tissue as diagnostic of myelocytic cells, studied the distribution of the oxidase reaction in a myeloma by means of a reagent consisting of 1 per cent α-naphthol in a 1 per cent solution of KOH with 1 per cent dimethylparaphenylendiamine. They observed color formation in the greater number of the cells of which the tumor was composed. Boots (2), working in this laboratory, obtained a positive reaction in a case of chloroma with the Graham modification of the oxidase reagent but a negative finding when he employed the Schultze technique.

Graeff (3) in 1912, however, had mentioned the occurrence of oxidase granules in adenoma and carcinoma (page 380) though he failed to find any trace of the reaction in a spindle-cell sarcoma of the kidney. In the same article, he adds that von Gierke had personally communicated to him the finding of a distinct oxidase reaction in the cells of mouse tumors.

Recent work on normal histological material by several investigators has demonstrated the wide-spread distribution of the oxidase reaction. The occurrence of the reaction in varying degrees in practically all normal tissues of the body suggests its application to pathological material. Further, since the reaction has been shown to be an adsorption phenomenon, which is dependent on such factors as the composition and potential of the various reacting components, it was thought possible that a study of tissue from neoplastic growths might yield some data concerning variations of the surface properties of the cells or their constituent elements.

TECHNIQUE

The material used in this study was derived from surgical specimens removed at operation. These had been preserved in 5 per cent formalin and form part of the pathological stock material. In a few instances results were checked by sections made from recently obtained material. By repeated washing in distilled water the excess of formalin was removed from the blocks of tissue, which were cut by the freezing microtome in sections varying in thickness from 5 to 10μ. These sections were then immersed for varying periods of time in a reagent composed of an aqueous solution of M/2000 α-naphthol and M/2000 paraphenylendiamine. The details of the staining reaction and technique have already been discussed in a previous article (4). It may not be amiss, however, to emphasize again the precaution of keeping the amount of tissue small in comparison to the amount of reagent in which the sections are immersed. As already noted, formalin fixation has no appreciable deleterious effect on the development of the oxidase reaction in the tissue. However, Zenker's fluid alters the tissue so greatly that after fixation in this medium the reaction fails entirely or obtains only in a very small degree. Not infrequently as a result of formalin fixation, deposits of unknown composition are encountered in the sections, and as these may sometimes interfere with the recognition and interpretation of a positive oxidase reaction, unstained sections were always mounted in the later part of the work for purposes of comparison with the stained. Sections were mounted in a 50 per cent aqueous solution of glycerin. The tissue thus treated can be preserved fairly well for several months. The time of immersion required to produce color reactions varies usually from three to twenty hours, though changes in the functional activity of the cell, as will be pointed out subsequently, exert a marked influence on the rate of reaction, so that frequently one to two hours suffices to produce a maximum color. We have restricted our study to well-defined types of neoplasms in which there was no question concerning the diagnosis.

TUMORS ARISING FROM CONNECTIVE TISSUES

Corresponding to the numerous modifications occurring in the morphology of normal connective tissues is an almost equal number of types of tumors arising from connective tissue. We have studied the oxidase reaction in sarcoma, fibroma, lipoma, osteo-chondroma, and myxoma. That normal connective tissue does not readily react with the oxidase reagent is indicated by the negative findings of Wolff (5) on connective tissue of placenta when sections of this organ were stained with a 1 per cent aqueous solution of α-naphthol and paraphenylendiamine and by the somewhat contradictory statements of von Gierke (6) and his pupil Graeff (3) regarding the ability of connective tissue to stain with the above reagents when dissolved in normal salt solution. von Gierke, using a combination of these two substances in 0.75 per cent concentrations, reports a positive finding, while the latter, with a more dilute concentration of the above reagent, failed to obtain any reaction except an occasional granule at the edge of the nucleus. Graeff believed the presence of a positive reaction to be dependent on the age of the cell. As has been previously shown by us the oxidase reaction does not readily develop in connective tissue in a watery solution of these substances. After an immersion varying from ten to twenty hours in an aqueous mixture of M/2000 α-naphthol and M/2000 paraphenylendiamine (and it should be emphasized again that α-naphthol is not soluble in cold aqueous solution above M/2000 concentration and even at this concentration occurs in the colloidal state) a blue color obtains in the protoplasm lying immediately about the nucleus, and especially at either pole. This blue color is due to the presence of fine granules. Coincident with the staining of these perinuclear granules there was found a blue staining of the nucleolus and frequently, though not always, the chromatin knots showed a faintly positive reaction. The color produced in these perinuclear granules may be considerably varied by modifications in the reagent, and can be enhanced by the addition of certain salts in definite proportions. Thus using the Sörensen phosphate series as a solvent for the α-naphthol and paraphenylendiamine, it was previously shown that these perinuclear granules reached a maximum size and number and developed the most intense color at a concentration corresponding to about P_H 7.46. Undoubtedly, not only the composition of the media bathing these cells but also their functional activity exerts a considerable influence on the degree of the reaction. As color development depends upon factors governing adsorption, fixation of tissue as well as concentration of reagents and composition of its solvent will modify its rate. In the normal connective tissue cells these perinuclear granules possess some degree of lability. This property is more evident in certain tumors of connective tissue derivation.

Sarcomata

The reaction as a whole obtaining in sarcomata is characterized by the negative or relatively slight reaction in the nucleus and the contrasting degree of the stain occurring in the granular cytoplasm immediately surrounding the nucleus. In actively growing spindle shaped cells in fibrosarcoma there occurs a considerable deposition of the indophenol blue upon the perinuclear granules and especially in the region of the poles of the nucleus. This granular material appears to be somewhat greater in amount than in the normal cell and in many of the tumor cells is found extending in cone like formation almost to the spindle ends of the cells. Between the nuclear wall and these stained granules, which are relatively fine in size, no clear zone can be discerned. Indeed, the reaction is most intense in the cytoplasm in the region immediately contiguous to the nucleus. The nucleus in these actively growing fusiform cells usually gives no reaction whatever though occasionally a very faint violet tinge may be observed in the nucleolus. The nuclear wall appears as a mauve outline, but definite granules cannot be distinguished. I have never been able to recognize chromatin threads or knots in the nuclei of these cells. The contrast between the practically colorless nuclei and the mauve granular cytoplasm is striking. In the cells with the nuclei of a more spherical form the granular reaction is fairly uniformly distributed in a narrow zone about the periphery of the nucleus. When giant cells occur in the sarcoma the reaction is deeper in color than in the smaller cells and it is almost impossible to distinguish individual granules because of their close aggregation. Frequently this colored granular material is so densely packed as to obscure the nuclei, which are colorless. It is not clear whether the greater intensity of color results from an increased number of granules within the cell, or from a deeper color of individual granules.

The most remarkable reactions which occur in sarcomata, however, are to be observed in those cells which through lack of adequate blood supply show degenerative changes. Under these altered conditions of environment and nourishment the perinuclear granules have become so altered in composition and surface properties that with a short immersion in the oxidase reagent one obtains colored granules which rival and even surpass in intensity those found in the myelocyte. These degenerative changes can be followed most readily in the spindle shaped cells. The alteration in the staining qualities of the cell appear first in the granular protoplasm about the poles of the nucleus, where the degeneration manifests itself by an increase in the size and number of the granules, which also assume a more intense violet color. As the degeneration process progresses, the color gradually increases in intensity and finally becomes blue-black. Through a coalescence of the smaller particles there result many large granules, which spread not only out toward the pointed ends of the cells but may also extend over the region of the nucleus. Coincident with these cytoplasmic changes the nucleolus takes on a bluish tinge, and finally stands out as a dark blue mass. Fine chromatin lines with irregularly distributed deeper masses representing chromatin knots can also be distinguished. The nuclear wall has become much deeper in color and definite granules appear on its surface. It is difficult to determine whether or not the nucleus participates in the formation of these extra-nuclear granules. Finally the whole cell may be filled with these deeply blue stained masses of varying sizes, many of which have become incorporated into large globules which appear to possess a semi-fluid consistency. The Brownian movement which these particles possess is very apparent in these large globules. The oxidase deposit occurs only on the periphery of the large globules. In the cells of oval and circular contour the degeneration may begin at any point, and progress to the final stage in which the cell protoplasm has been largely converted into a mass of colored globules. These globular masses can be recognized in the formalin fixed unstained frozen sections, and can also be stained with neutral red. They appear to be identical with the granules in fibroblasts grown in vitro, which have an analogous distribution and which have been recently described by Lewis (7). The intimate association of these granules with the centrosomes and mitochondria has already been noted by that author. In melanosarcoma the granules giving the oxidase reaction are quite distinct from the melanin pigment of the cell. The degenerative changes just outlined, which are accompanied by the increased affinity for indophenol, may develop in these pigmented cells and apparently have no relation whatever to the melanin granules. In lymphosarcoma the nucleus is relatively free from reaction and fine deep blue granules appear irregularly scattered throughout the cytoplasm. The appearance is similar to that previously described by us for the normal lymphocyte. When the sarcoma cells in the course of their development give rise to intercellular collagen, this substance stains a diffuse pink with the occasional formation of more deeply reacting longitudinally arranged fibrils. Besides the changes in the sarcoma cells, others may occur in the same sections throughout the normal tissue; these consist of large deposits of deeply stained material resembling fat and occupying the connective tissue framework. Occasionally these may be displaced leaving cavities. These globular masses stain on the periphery only and not infrequently the center consists of numerous unstained white acicular crystals.

Fibromata

Oxidase reaction was studied in six fibromata, all of which had arisen in the skin. Like sarcomata, these are characterized by the very faint reaction occurring in the nucleus, and the more intensely staining zone of perinuclear granular material. Two of the tumors were of the *fibroma durum* type. In these the nuclei were small and the perinuclear granular protoplasm much reduced in amount. The nuclei showed no reaction except for a very faint mauve coloration of the nucleolus. Lying about the nuclei and extending longitudinally outward from either pole as a thin line there is a very intensely stained, blue-violet, granular protoplasm. This narrow strip lies between thick parallel bands of collagen which for the most part is homogeneous and stains a pale pink, although in some areas it may show the presence of faintly mauve colored granules, which occasionally are arranged in fibrils lying parallel to the long axis of the nucleus. Other histological structures occurring in the skin, e.g., sweat and sebaceous glands, hair follicles, and malpighian layer give a very intense reaction.

In the other four tumors, which represent various degrees of the soft type, the cells are larger and the matrix less in amount and in these the reaction approaches more closely that of the fibrosarcoma. The nucleolus can be distinguished as a mauve stained body, and occasionally chromatin knots may be faintly recognized. The karyoplasm stains a pale pink and under high magnification faint granules may be made out.

In two of the sections there occurs a considerable infiltration of lymphocytes and plasma cells, the cytoplasm of both of which shows numerous deeply stained moderate sized granules. Areas of these cells alternating with the more lightly reacting connective tissue elements afford a marked contrast. Degenerative changes comparable to those described in sarcomata are apparently not of frequent occurrence in fibromata, as in only one of the tumors studied was there any evidence of a degenerative process giving rise to those intensely reacting granules which are of such constant occurrence in sarcoma. The degenerative processes in the two types of tumors resemble one another closely. In fibroma, however, the nucleus appears irregular in outline and the nucleolus and chromatin knots assume a deeper stain at an earlier stage of the degeneration. The more deeply reactive globules appear first in the region bordering the poles of the nucleus. They may vary in size and may attain very large dimensions, and in the late stages appear irregularly distributed throughout the cell contents. Occasionally these deeply stained spherical globules may be extracellular.

Myxomata

Only two cases of myxoma were studied. The sections consisted of a pink stained homogeneous matrix, in which were irregularly scattered many cell bodies with fine thread-like prolongations ramifying in all directions.

The cell body consisted of numerous deeply stained granules embedded in a homogeneous mauve protoplasm. The granule formation extended outward into the prolongations, which appeared of a deep blue color owing to the fact that the granules lay closely packed together. The nucleus showed no reaction except for a faint diffuse mauve color in the nucleolus which was often obscured by the wealth of overlying deeply stained granules.

Osteo-chondroma

Only two sections were studied. At the periphery of the tumor, where proliferation was active and where the cells were fusiform in outline, the reaction within the cell was most intense at either pole of the nucleus, over which it extended like a cone. The cytoplasm consisted of a pale mauve matrix in which were embedded many deep blue granules. At the periphery of the cell in the region of the cell membrane these became denser and more closely packed together. The nucleus showed a fairly well stained nucleolus, but it was not possible to distinguish chromatin.

In the zone lying next the periphery of the tumor, where the cells were still rapidly dividing, many cells were arranged in pairs with two sides in apposition as in normal cartilage. In these the reaction was similar to that occurring in the cells already described. The matrix in which these cells lie was of a faint homogeneous pink color. In the more mature parts of the tumor the cells were larger in size and the granules lying in the cell body became coarser, more deeply stained, and frequently assumed a concentric arrangement about the nucleus, where they sometimes occurred in such close apposition that it was difficult to recognize individual granules. In the same fields many cells were also found in which the ground substance of the cell had become almost colorless, but throughout this colorless protoplasm were scattered many coarse deep blue granules. The periphery of both types of cell was formed by a narrow zone of coarse intensely stained granules. The matrix in which these cells lie was for the most part homogeneous and more deeply stained than near the periphery of the tumor growth. In certain areas it was converted into reddish stained fine fibrils, irregularly scattered through which one could find larger masses of deeply stained material.

Lipomata

The four lipomata studied belong to the subcutaneous type. In two of them the supporting stroma had been much reduced and the cells were so distended with fat that the tumors were difficult to section. In staining, the contents of the cells of the lipoma gave a reaction which could not be distinguished from that observed in cells of normal adipose tissue. In those cells where the conversion into fatty material had not been complete, the small globules of fat stained readily and appeared almost black in color. In cells which were so loaded with fat that the cytoplasm and nucleus could no longer be recognized, the lipoid contents of the cell stained on the periphery only and were of a deep blue-black color. In many of these large cells the central part was composed of long acicular crystals, which did not react with the oxidase reagent. In one of the sections orange yellow fatty deposits occurred, which stained much more slowly than the cells containing colorless lipoid material. The normal structures of the skin associated with the lipoma gave reactions similar to those found elsewhere in the skin.

Myomata

The myomata studied belong to the leiomyomata of the uterus. The oxidase reaction found in the muscle cells of the uterus occurs with greatest intensity in the undifferentiated protoplasm lying about the nucleus. This small area of granular protoplasm can be stained by other reagents and the granules have been demonstrated by Lo Cascio (8) with iron haematoxylin. Their staining properties apparently are closely related to their functional activity, for both Graeff (3) and Ikeda (9) found that in the muscle cells of the pregnant uterus the oxidase reaction developed more rapidly and with greater intensity than in the muscle cells from the normal organ. These observations on the variability of the reaction with the oxidase reagent we have been able to confirm and have noted marked differences between the staining of the myometrium from different preparations. It may vary from a mauve reaction in which the granules can not readily be recognized, to distinct granules with a maximum blue-black color. In one case, in which an adenocarcinoma had invaded the myometrium, a few minutes following immersion of sections in the oxidase reagent the appearance of blue color was observed in the perinuclear granules of the muscle cells in areas irregularly distributed, and at the end of from two to three hours a reaction equal in intensity to that of the myelocytes had developed. It has not been possible thus far to determine definitely the factors underlying these variations. The fact that they do not occur uniformly distributed, and that the areas in which the reaction is most intense are bands of myometrium which have become segregated by the actively growing cancer cells, suggests decreased metabolism through pressure to be a predominating factor.

We have found that in myomata the perinuclear granules always show a pale mauve color at the end of five or six hours, and at the end of twenty hours stand out fairly distinctly. Simultaneously with staining of the cytoplasmic granules, color appears in the nucleolus and chromatin knots. The distribution of the extra-nuclear oxidase reaction seems to depend largely upon the shape of the cell. In the spindle shaped cells these granules lie as in the similarly shaped connective tissue cells, aggregated about either pole of the nucleus. They may lie in close apposition to the nucleus or may be separated from this structure by a narrow clear zone. In these cells the granules are usually of fairly large size and are intensely stained. While the distribution is similar to that of the fusiform connective tissue cells, the two can be differentiated by the difference of the intensity of the reaction of the perinuclear granules and of the nucleus. In the connective tissue the reaction in the nucleus is almost negative in comparison to the decided mauve color of the nucleolus and chromatin material of the muscle cell, and in normal tissue the perinuclear granules of the muscle cell always stain more deeply when immersed in the same reagent for like periods of time than the corresponding granules of the connective tissue cell. When the cells and nucleus approach the more circular form or are oval, the extra nuclear material appears uniformly surrounding the nucleus. This granular material may lie contiguous to the nuclear membrane or may be separated from it by a clear zone. The amount of this material is never very large and may be present in amounts varying from a few granules to 20 or 30 or to a cloud of intensely stained fine granules. Graeff (3) has also noted considerable variations in the number of the perinuclear granules of the normal muscle cell which give the oxidase reaction.

The granules vary in size and in some of the sections examined granular formation could only be distinguished under high magnification. When fibromatous elements are present in the myoma, the longitudinally arranged collagen bands reveal a pale pinkish coloration, in which occasionally fine fibrillation can be seen. The reaction in the nucleus consists of a number of irregularly scattered, large sized, well stained granules. The nucleolus also stains deeply. In some of the preparations the granules are imbedded in the nuclear wall, and they vary somewhat in size. The reaction appears first in the nucleolus but also develops early in the chromatin knots.

Myeloma

Only one myeloma was available and this was of the myeloid type. The gross specimen, which consisted of various sized masses of soft yellowish brown material was curetted from the upper part of the tibia. On microscopic examination the section was found to be fairly vascular, and composed of closely packed medium sized cells with a large clear nucleus and a comparatively small amount of cytoplasm. Irregularly scattered throughout the section also were giant cells, containing from three to ten nuclei. These cells gave the sections a very characteristic appearance. They exhibited a marked affinity for the indophenol, showing the presence of a definite bluish color following an immersion in the reagent of from one to two hours' duration. At the end of seven or eight hours they stood out as intensely blue stained masses. The cytoplasm was homogeneous and mauve in color and in it were embedded deep blue granules of varying size, which were closely aggregated. The nuclei were colorless and morphological detail was obscured by the overlying granular material.

The smaller cells had colorless nuclei. The cytoplasm also was colorless, and contained coarse deep blue granules, in the larger of which Brownian movement could be detected. In addition to the oxidase granules these cells occasionally showed the presence of globular masses of yellowish material, which did not react with the reagent.

Endotheliomata

In a previous paper we (4) reported a very slight pink reaction obtained in the endothelium of blood vessels, a finding which is in accord with the observations of von Gierke (6) and Graeff (3). Though more than twenty tumors of blood-vessels have been examined, among which were endotheliomata and lymph- and haemangiomata, we have invariably found that the endothelial cells of these growths also exhibit a negative or faintly positive reaction.

TUMORS DERIVED FROM EPITHELIAL TISSUES

Normal epithelium everywhere throughout the body reacts to a greater or less degree with the oxidase reagent. The color formation which is obtained varies with the organ and the part of the organ in which the epithelium occurs, as well as with the physiological activity. Thus Klopfer (10) reports various gradations of the indophenol synthesis in different parts of the kidney tubule, and Lillie (11) finds that while the epithelium throughout the intestinal tract gives a positive reaction this is most intense in the stomach. Similarly we (4) have observed the most intense reaction of squamous stratified epithelium in the skin. The influence of functional activity upon the oxidase reaction is well illustrated by the varying degrees of intensity obtaining in the secretion granules during the different stages of the secretory process in the intestinal tract. The same wide variation found in normal epithelium is also found in tumors of epithelial derivation. It is noteworthy that it is those tissues showing wide variations in the oxidase staining capacities in which the cancer incidence is high. That is, tissues in which the lability of the granules (as measured by differences in the oxidase reaction) is marked, are those in which tumors are prone to occur. Among these one may especially mention stratified squamous epithelium and epithelium of the intestinal tract and the uterus.

The oxidase reaction has to do primarily with the surface properties of protoplasmic granules and one may obtain the same picture in cells from tumors which vary widely in structural characteristics. Cells in various areas of the same tumor may show the widest divergence; cells from different types of tumors in the same or in different organs may appear identical in staining qualities. Thus while considerable difference occurs in the morphology of papillary and adenocarcinoma the cytology of the two may be indistinguishable.

As has been already indicated, the range of the oxidase reaction within different organs or different parts of the same organ is not the same. In normal structures this range of intensity is largely dependent upon the intrinsic constitution of the part and its physiological activity. In the tumor one recognizes that in addition to these two factors the age of the cell is also a potent influence in modifying the development of color. That is, in cancer, the intensity of the oxidase reaction closely corresponds to the stage of cell development, being minimal in the newly formed cell and gradually increasing until a maximum is reached in the old degenerating cell. Thus, in the peripheral parts of the cancerous growth, regardless of its structural peculiarities, the outstanding characteristic is the ability of the rapidly dividing cells to inhibit the indophenol synthesis compared with that displayed by the cells of the maturer, better developed tissue. It is evident from the above that any very detailed account of the oxidase reaction in carcinoma would consist largely of a description of color reaction of varying intensities. As this study deals with reactions in individual cells rather than structural differences, to avoid repetition and make descriptions as concise as possible we have arbitrarily divided cancer into two groups according to whether they are derived from stratified squamous epithelium or glandular epithelium.

Carcinoma Derived from Glandular Epithelium

Under this heading are included growths derived from the epithelium of the intestinal tract below the oesophagus (consisting mostly of those of stomach and rectum) and from the uterus and breast. They vary in architectural structure and may be of adenomatous, papillary, or diffuse types.

Where the tumor is actively proliferating the cells appear of a very pale mauve color. This is partly due to the fact that the nucleus, which is large in proportion to the cytoplasm, is very faintly stained. The nucleolus is barely discernible and the remainder of the nucleus is made up of a network of very faint mauve lines in a colorless karyoplasm. The nuclear membrane is seen as a thin, deep blue line. The cytoplasm is scant and is filled with granules of a faint reddish mauve color. As the cells become more mature the granules increase in color and the nucleolus and chromatin threads become more prominent, taking on a bluer tinge. As the cells tend towards degeneration the nucleolus assumes a swollen appearance and becomes brownish in color, and the chromatin threads remain fairly well stained.

The gradual increase in size and color of the protoplasmic granules is well illustrated in the goblet cell where, as the mucous secretion accumulates, the granules gradually become larger and acquire a greater affinity for the stain. In the rectum one may occasionally find accumulations of these deep blue granules lying immediately over the mouths of the goblet cells. The nucleus shows a faintly stained chromatin network with a fairly deep mauve nucleolus. When the growth, becoming highly invasive and, losing its adenomatous character, grows diffusely, as in the uterus for example, the granules in the cytoplasm may stain a deep blue and be so numerous as to obscure the histological details of the nucleus. In the breast, when the adenomatous character of the growth persists, the gland epithelium contains a few coarse granules near the base of the cell, where the nucleus occurs, but a wealth of fairly coarse granules occur within the free border. The connective tissue, when it has not been encroached upon to any extent by the surrounding neoplasm, shows a fairly normal reaction. When the tumors, however, proliferate so rapidly as to give rise to pressure upon the interstitial tissue, the perinuclear granules of the connective tissue cells may show a very distinct deep blue reaction, due to the formation of degeneration granules. This phenomenon is common in tumors of the breast, but is exhibited in its greatest extent in the colloid cancers of the stomach. In rapidly proliferating tissue in which the nuclei became much enlarged and contained chromatin in clumps, we have noted that these latter exhibited a marked affinity for the indophenol and appeared as blue-black masses.

In those adeno-carcinomas of the stomach and rectum which are designated "colloid cancers" because of the hypersecretion and subsequent accumulation of mucin in the alveoli, the substance secreted undergoes a change in its staining qualities subsequent to its expulsion from the cell. As it lies just outside the lumen of the gland cells it consists of many very deep blue granules embedded in a pink homogeneous material. When the alveoli have become distended with this mucous secretion, however, the granules tend to disappear and the matrical substance to become colorless. This decoloration is most apparent in the central part of the alveoli.

Squamous Cell Cancers

In general the oxidase reactions obtained in the stratified squamous epithelium which has acquired carcinomatous properties closely resemble those found in the normal epithelial tissue of a similar type. This layer, not only where it is limited to a normal distribution, but also where its outgrowths have invaded the underlying tissue, stands out in sharp relief against the more faintly reacting contiguous connective tissues as a deep blue mass. In areas where keratinization has taken place, both in stratum corneum and in the epithelial pearls, the oxidase reaction reaches its maximum intensity and appears as a dense blue-black mass. It may be so intense as to obscure all morphological detail.

In contrast to this the stratum lucidum when present shows a very pale reaction. Within the epithelial growth the constituent cells in the various strata exhibit considerable variation in staining qualities. At the growing tip of the cancer mass which is extending into the surrounding tissue, the reaction assumes a fairly intense diffuse color and especially in those cells which are rapidly dividing. This intensity of color is due to closely aggregated bluish-red granules in the homogeneous mauve cytoplasm, which is small in amount. The nucleus is comparatively large, and is clearly outlined by a fine deep blue nuclear membrane. In the colorless or faintly pink staining nuclear contents can be recognized the nucleolus, which is fairly large and is colored a diffuse mauve. Occasionally the chromatin knots appear faintly stained.

In the zone lying beneath that actively proliferating, the cells are larger; and while the individual granules are deeper in color because they are here not so closely aggregated, the whole cell may not show any increase in intensity of stain. In the layers more remote from the vascular supply the granules gradually become much coarser, of a deep blue color, and stand out very distinctly. The volume of the cells may be much increased, the nucleus is larger, and the nucleolus appears swollen and frequently may reach three or four times its original volume. It is much paler in color and usually appears to have a yellow-brown tinge. A chromatin network can be faintly distinguished.

As the cells become still more flattened and compressed the granules become progressively larger in size until at the centres of the epithelial pearls the entire cell contents are converted into large clumps of deep blue-black material. Occasionally what corresponds in staining qualities to the stratum lucidum may intervene beween the flattened keratinized cells in the centre of the epithelial pearls and the zone lying peripherally. These cells contain an almost colorless ground substance in which occur rather sparsely distributed granules, which stain a pale mauve. The nucleus is irregular in outline and almost colorless except for the presence of a large diffusely pale stained nucleolus.

It is of interest to note the reaction occurring in the fibroblasts, which have been stimulated to proliferation by the extension of the neoplastic cells into the connective tissue. Unlike the reaction usually found in the adult connective tissue or in fibrosarcomata, not only are the perinuclear granules deeply stained, but the chromatin as well as the nucleolus is stained. This increased color reaction is not infrequently observed in epitheliomata of skin and it is possible that pressure is a factor in its production. One also finds in these neoplasms structures which may occur in normal squamous stratified epithelium. Intercellular bridges when they occur stain a very deep blue in contrast to the pink homogeneous cement substance between the cells. These cellular connections are made up of very fine granules lying in close apposition. Further the fibrillation which is sometimes seen in the normal cell may be observed passing through the cytoplasm as parallel bands of fine blue lines.

Karyokinetic figures are never to be recognized as distinctly stained structures, but occasionally appear as faint shadows. In places collagen formation may occur, showing the usual pale pink stain with compressed deeply reacting perinuclear substance in the connective tissue cells lying between the longitudinal collagen bands. In the skin it may also be noted that where splitting of the elastic tissue occurs these fibres take on a diffuse deep bluish purple stain. An observation of interest was the deep reaction occurring in the medullary sheaths of the cutaneous nerves.

DISCUSSION

As has been frequently stated in this and in a previous paper on oxidases, if one employs a suitable combination of α-naphthol and paraphenylendiamine the oxidase reaction can be demonstrated in every tissue in the body, though not with an equal degree of intensity in all. As color development in the indophenol synthesis is due to oxidation it will be necessary at the outset to consider briefly the formation and composition of indophenol and part at least of the kinetics concerned in the production of its color, in order to obtain a better understanding of the staining process occurring in the tissues. As was indicated previously both α-naphthol and paraphenylendiamine form colloidal solutions in water. The colloidal particles of the former acquire in this medium a positive electric charge while the paraphenylendiamine becomes negatively charged. The sign of the charge on the colloids was determined by observing migration of these substances in a Michaelis (12) kataphoresis tube when an electric current was passed through the solution. Since the color of either substance alone in an aqueous solution is so slight, even when the electric current has been passing through a solution of one of them for a fairly long period of time and has effected the transport of a considerable amount of the colloid towards one pole, its presence in the region of this pole cannot be recognized by its color alone. If, however, the lower stopcocks of the apparatus are closed and the electrode tubes are carefully removed, addition of the same amount of paraphenylendiamine to the solutions in both arms of the U-tube, when α-naphthol is being studied, will indicate by the speedy development of color into which arm of the U-tube the colloid has migrated. Similarly if the migration of paraphenylendiamine is being investigated its charge can be determined by reinforcing the color by the addition of α-naphthol in equal amounts to both limbs of the U-tube subsequent to the passage of the electric current.

In aqueous solution, then, α-naphthol is an electro-positive colloid and paraphenylendiamine is electro-negative. Now if these two solutions are mixed, through the agency of surface condensation there will result a new colloidal complex whose composition will depend in large degree upon the amount of neutralization occurring between the two oppositely charged colloids, which is determined by their concentration and degree of dissociation.

When these two colloids balance each other electrically a neutral compound will be formed and slowly precipitate out of solution. With this neutralization is associated development of color, as can be well demonstrated by employing the Sörensen phosphate solutions as solvents. When equal parts of the two substances composing the reagent are added in equimolecular (M/2000) proportions to the various solutions of the phosphate series, this electrically neutral compound, indophenol, is formed in greatest amount in the solution having a value of P_H 7.17, the so-called iso-electric point, since in the kataphoresis tube the colloid in a solution of this reaction moves to neither pole. At this point the maximum color develops. In the more acid or more alkaline solutions there is a definite migration to the positive and negative poles respectively and a corresponding decrease in color. In the solutions which are more alkaline in reaction than P_H 7.17 the diminution in color is less rapid than in the more acid solutions and little decrease in intensity is noted up to P_H 8.04. In aqueous solutions indophenol has a negative electric charge which is capable of being neutralized by surface condensation when contact with oppositely charged tissue constituents occurs. This is accompanied by increased development in color.

Now when sections are placed in an aqueous solution containing M/2000 α-naphthol and paraphenylendiamine the various tissue components acquire a negative or a positive charge or, if at their iso-electric point, no charge whatever. If sufficient ions are discharged into the water to alter its reaction to such an extent that the indophenol no longer reacts as a negatively charged, but has been converted into a positively charged colloid, oxidation does not occur and practically no color develops. This happens when the amount of tissue is large compared with that of water. If however a comparatively small amount of tissue be placed in the reagent the formation of the indophenol blue in the tissue may be interfered with by a separate combination of either one of the reagents with the tissue components, or if the indophenol is formed, subsequent to its adsorption by the substances in the tissue, oxidation or reduction may take place. If the former process (i.e., separate reaction of the α-naphthol or paraphenylene with the tissue) occurs it is apparently not associated with the development of color in the tissue, for if one transfers the sections every hour to a freshly prepared reagent the development of the color is delayed indefinitely.

If, as in the second case, the indophenol is formed, whether or not color develops will depend upon the charge of the tissue component adsorbing this dye. If this is negative, reduction of the reagent or transfer of positive valence from it to the tissue occurs and no color is formed. If on the contrary the tissue is positively charged, there results through oxidation (or increase in positive valence of the reagent) indophenol blue, the amount of which depends upon the degree of dissociation of the adsorbing substance in the tissue.

Thus far we have discussed surface charge as if it were the only factor concerned in adsorption of indophenol. By means of a Traube stalagmometer it can be shown that the surface tension of a solution of α-naphthol is lowered on the addition of the paraphenylendiamine upon the formation of indophenol and its oxidation. While surface charge and surface tension are intimately related there is reason to believe that surface tension, per se, is an important factor in the formation of indophenol blue in the tissues. However, the relation of oxidation, which in its broad concept is analogous to an increase in the positive electrical charge, to alterations in color makes the connection between oxidation and surface charge easier of demonstration, than a definite relationship between oxidation and surface tension. The production of a marked color reaction upon immersion of normal tissue in the oxidase reagent in stratum corneum, fat globules, eosinophiles, neutrophiles, and decidual cells marks these as tissues possessing a common property which is related to electrical dissociation. The electro-positive character of fat globules and the granules of the stratum corneum is not so prominent as their low surface tension. An evidence of the close relation which this latter property bears to the formation of a positive oxidase reaction is seen in the markedly enhanced reaction occurring in the lipoid substances of the medullary sheaths following the exposure of peripheral nerves to ether, chloroform, bile, and various other fat solvents which lower surface tension.

In contrast to the above enumerated tissue constituents showing a marked positive reaction are those in which a minimal reaction obtains. Among these one notes the nucleus preeminently but likewise the matrix of cells, collagen, and endothelium. Between these two extremes lie tissues in which all gradations of color appear.

The intensity of the color reaction in a tissue is thus a direct index of the amount of dissociation giving rise to a positive surface charge which the indophenol is capable of abstracting from the tissue. That is, the color reaction is a measure of the degree of reduction of tissue effected by the indophenol (or, what amounts to the same thing, is an inverse measure of the oxidative capacity).

Under certain conditions the reaction may also yield further information. It may constitute a gauge of the inherent potentiality of the tissues as well as the conditions and approximate rate by which this potential may be transformed into kinetic energy. This is illustrated in the variations of the reactions described in sarcoma. In the connective tissue cell under changes of environment, among which increase in the hydrogen ion concentration of the surrounding media is prominent, the finely granular positively charged perinuclear material is converted into colloidal complexes with new physico-chemical properties. The original granules, probably through the adsorption of other colloids, have been finally reduced to large globular masses with a low surface tension and considerable electrical charge. At this stage the oxidation power of the indophenol is high compared with that of the tissue granules and color formation in the former reaches a maximum.

By means of this reaction it can thus be shown that these perinuclear connective tissue granules not only possess a high potential energy, but that this energy is capable of being readily transformed under pathological conditions into the kinetic form of energy, which readily reacts with the indophenol. Similar physiological transformations are also encountered in the conversion of the granular contents of the basal cells of the malpighian layer into the intensely staining eleidin and keratohyalin. It has even been postulated by Tettenhamer (13) though without very convincing proof, that in this instance the alteration actually includes the chromatin of the nucleus. It is an interesting coincidence that the tissues in which the lability of the granular contents, as demonstrated by the oxidase reaction, is most marked, are those in which the regenerative capacities are highest. While this lability of granule can be most readily demonstrated when it results in an increased oxidation of the oxidase reagent (or conversely a reduction of the tissue component) there is unmistakable evidence of its existence in the opposite direction. That is, an accumulation of potential energy is manifested in the nucleus and perinuclear material of actively proliferating tissue by the diminution in the intensity of their oxidase stain. Instances of nuclear change represented by increased development of color, and interpreted as a decrease in potential energy have also been described throughout the paper.

In conclusion it is to be emphasized that all the color reactions discussed in this paper are always in relation to the iso-electric point of the indophenol, which is fundamental, and hence the surface charges are not absolute but relative. Therefore whether oxidation or reduction predominate in tissue or reagent, depends upon the physico-chemical properties of each, which is determined in no small degree by the character of the media in which they occur. A slight alteration in the environment of amphoteric substances, especially in the vicinity of their iso-electric point, will induce far reaching changes in their reactions, as is attested by the conversion of positive into negative oxidase reactions, or vice versa, by the addition of traces of an alkali to the reagent.

SUMMARY

1. Development of color in the reagent is due to oxidation. Therefore, when the adsorption of the reagent by a tissue is followed by an increase in the intensity of the color, increased oxidative capacity has been developed in the reagent. This involves a corresponding tissue change in the nature of a reduction.

2. The more marked the oxidase reaction the greater the reduction occurring in the reacting tissue, and vice versa.

3. Cell constituents of certain tissues, in which a slight oxidase reaction normally develops, may through degenerative processes be converted into new colloidal complexes in which a maximum reaction obtains. Examples of such alterations in intensity of reaction are found in connective tissue (spindle-cell sarcoma) and in stratified squamous epithelium (epithelial pearls of epithelioma). These variations are associated with a transformation of potential into kinetic energy and indicate a considerable degree of lability of granules.

4. It is a noteworthy coincidence that this lability of cell granules, as measured by variations in the oxidase reaction, occurs in tissue with a high regenerative capacity.

5. In the nuclei, intensity of color reaction may be increased or diminished. The first occurs in degenerative processes, the latter is observed in cells which are rapidly proliferating.

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THE PEROXIDASE REACTION IN THREE CASES OF MULTIPLE MYELOMA OF THE BONES WITH REMARKS CONCERNING THE NOSOLOGICAL POSITION OF THESE TUMORS

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The exact characteristics which allow a tumor to be admitted to the myeloma group have never been uniformly agreed upon in pathological literature. From a clinical point of view the cases are fairly well defined. The presenting symptom which brings the patient to the clinic is usually pain of a constant, distressing and deep seated character, associated with marked weakness and cachexia, and occasionally with complaints leading the physician to the consideration of organic disease of the spinal cord. Physical examination shows the emaciation, loss of weight, and an anemia usually of severe grade. Careful and detailed inspection of the osseus system reveals the bone tumors usually most evident in the ribs, but often found in the long bones by the presence of pain or spontaneous fracture. The *x*-ray leaves no doubt of the condition, since the finding of circumscribed or diffuse bony tumors in practically all parts of the body makes the diagnosis. The Bence-Jones albumose may or may not be found in the urine. The relatively rare occurrence of this disease along with the present hopeless prognostic outlook has caused interest to be centered chiefly in the histological pathology of the tumors and the discussion of their nosological relationships.

The name multiple myeloma was first applied by v. Rustizky (1), who regarded the condition as rather a hyperplasia than a neoplasm and who believed that the characteristic cell was a marrow cell. This stand was taken because of the general resemblance both gross and microscopical which the tumor bore to marrow tissue, rather than as a result of detailed microscopical study of the cellular elements involved. Indeed at this time the detailed histology of the marrow had not developed to a degree to make this sort of study possible.

Cases of myeloma had been described previous to v. Rustizky's account under various names, but taking the literature as a whole both preceding and following 1873, it becomes immediately evident that if many cases are to be admitted into the myeloma group we must either interpret the term liberally to mean multiple tumors in general associated with marrow, or assume the differences of description and interpretation to result from the undeveloped state of histology at that time. Similar or related clinical conditions and pathological findings are described under many names.[1]

Several important considerations relating to classification constantly arise throughout the series.

One of the earliest of these was whether the disease should be considered as a neoplasm or as an inflammatory or hyperplastic reaction of normal marrow elements. Rustizky (1) in his original description speaks of the condition as a hyperplasia rather than a heteroplasia and separates the condition from the myelogenous sarcomata of Virchow (13) on this point. Grawitz (2) reports three cases under the name osteomyelitis maligna which were probably not myelomata but aleucemic or chloromatous in nature. Abrikossoff (14) believes the cells to be of myelocyte type and discusses the position of myelomata relative to hyperplasia and neoplasia. Wieland (3) reserves the term myeloma for those new growths that are myelomatous in the anatomical sense, that is, whose structure does not depart from the mother tissue. Abrikossoff is not able to agree with Kauffman (15) and Wieland that a separation into myeloma on the one hand

[1] Myeloma multiplex (Rustizky) (1), Osteomyelitis maligna (Grawitz) (2), Lymphosarcoma multiplex ossium (Wieland) (3), Sarcoma multiplex ossium (Buch) (4), Pseudoleucaemia myelogenes (Runeberg) (5), (Zahn) (32), Ostitis sarcomatosa (Hammer) (6), Lymphadenia ossium (Nothnagel) (7), Endothelioma intravasculare (Markwald) (8), Myelosarcoma (Schmaus) (9), Erythroblastoma (Ribbert) (10), Plasmoma malignum (Hoffmann) (11), Myeloblastoma (Symmers) (12).

and multiple lymphosarcoma on the other is possible. He thinks the essential point is the inability of the myelomata to metastasize and compares them to lymphosarcomata of lymph-nodes (aleucemic leucemia?) in this respect, but separates them sharply from ordinary bone sarcomata. Since there are several undoubted myelomata now in the literature which have shown a certain degree of metastasis, Abrikossoff's basis for his conclusion is done away with. Relating to this question, it is of interest that, as time has gone on and the conception of neoplasia has become more fully developed, this matter has settled itself, and myelomata are admitted into the neoplasm family with the same doubts and reservations and the same watchful waiting regarding their ultimate disposal as is the case with the leucemias.

The main subject for settlement, however, has been the relation of these tumors to the myelocyte series of marrow cells.

The possibilities presented are obvious. These tumors might have their origin

1. From misplaced tissue not normally related to any tissue of the marrow.
2. From the ordinary connective tissue elements.
3. From the blood-vessels especially.
4. From fat tissue.
5. From lymphocytes.
6. From cells normally present and characteristic of marrow, but not part of the myelocyte series.
7. From the myelocyte series.

There is nothing in the life history or histology of multiple myelomata to suggest any such degree of heterotopia as the first consideration requires, and the possibility has never been discussed in the literature.

Before considering the remaining possibilities, the difficulty immediately arises as to what we mean by the term myeloma and how great a latitude regarding diversity of structure and power of metastasis we are going to allow to a tumor and still call it a myeloma. The disagreements between pathologists regarding the position in nosology of this tumor are always going to reduce themselves to this matter of definition until the matter of histogenesis is settled. For the present, the author believes that a liberal conception is the best one in that the definition should contain no dogmatic statements regarding the histogenesis, and that the condition of "multiple myeloma of the bones" should be defined as a multiple primary neoplasia arising in the bone marrow, showing marked ability to erode and destroy bone with little or no tendency to reparative process or callus formation, and with very limited powers of metastasis. The histology of the tumors is characteristic and quite definite and uniform in different cases as regards the picture presented under the microscope, in spite of the uncertainty and disagreement regarding the origin of the cellular elements. On this basis we can discuss the remaining questions.

The second possibility, namely, that we are dealing with tumors of ordinary connective tissue origin, can be discarded on the ground that in the first place the cellular content of myelomata is distinctive and not that of undifferentiated connective tissue. For sarcomata of a like degree of histioid structure they are far too benign and metastasize much too sparsely. Several characters set the myeloma apart from ordinary sarcomata; their multiple primary occurrence, their distinctive and uniform cell type, with a lesser degree of heteroplasia; their specific and marked ability to erode bone by lacunar absorption without producing a reparative reaction on the part of the bone, associated with a very low degree of ability to metastasize in spite of their local destructive character.

The same considerations, together with the fact that hemangenic endotheliomata are recognized to occur (Markwald (8), Berger (16), Thevenot (17), Symmers and Vance (18)) in marrow as a distinct condition in no way resembling multiple myelomatosis pathologically, disposes of the third possibility. In order to consider the fourth division we must assume that fat is a distinct tissue of separate ancestry from connective tissue, as Mallory has suggested. Beside the fact that this idea is not generally accepted, we have the limitation of myelomata to bones rather than a relation to fat tissue in general, and the fact that while there might be a fancied resemblance between the embryonic fat cell and the myeloma cell, the myeloma cells are more basophilic and do not differentiate like fat cells. In this connection it is of interest that Rustizky found cells in the tumors of his case that he interpreted as changed fat cells.

As to the arguments against a lymphocytic origin we are in the same position as we are with regard to fat. The myeloma tumors are always primary in bone, never[2] in lymph-nodes. They do not show even a predilection to metastasize to lymph-nodes. Moreover, multiple myelomatosis is not associated with abnormalities of the lymphatic tissue, and the tumor cells do not differentiate like lymphocytes.

On account of the fact that some cases reported as examples of myelomata have apparently been of the lymphocyte type, such as that of Herrick and Hektoen (19), there has been a tendency in the later literature to restrict the conception of myeloma definitely to those tumors having a characteristic histology. Thus Symmers objects to the cases of Herrick and Hektoen, Weber, Scheele and Herxheiner, and Kahler being included as myelomata. Symmers believes that apparent secondaries found outside the marrow usually arise by stimulation of latent centers in extramedullary hemopoietic viscera, but that true transplantation metastases do occasionally arise.

The differences in opinion as to which cases should be included as myelomata are well shown by the differences in the accepted lists of Symmers and of Pepper and Pearce (20).

The difficulty of sharply defining the limits of the myelomata has led many writers to make several groups. Thus Vance (21) distinguishes myeloblastoma, erythroblastoma, lymphocytoma, and plasmocytoma types. Christian (22), as heretofore mentioned, argues for the unity of the myeloma.

Pappenheim (23) considered them to be merely a special localization of processes belonging to the lymphosarcoma or pseudoleucemia group (Lymphosarcomatosis pseudoleucemica) and not a distinct entity in themselves.

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[2] Dr. Ewing in a personal communication states that there are a few cases of (myelomata?) arising in lymph-nodes.

Sternberg (24) admits four possible groups. His first and third groups are probably identical and would now be considered as myelomata.

Some growths have been diffuse in the bone marrow rather than represented by discrete nodules. Such cases are reported by Abrikossoff (14), Weber (25), Winkler (26), Kalischer (27), and Zochmann and Schumm (28).

Paltauf (29), Sternberg, and Kaufmann differentiate the primary multiple myelogenous sarcomata and lymphosarcoma from the group of true myelomata.

These were the easily settled questions which the literature has taken care of quite adequately and which have settled themselves as the cases have been reported.

There remain, however, two additional possibilities which are most important.

(1) Do multiple myelomata constitute a group by themselves having origin from a distinct if unknown series of bone marrow cells, or—

(2) Do they belong to the myelocyte series of tumors?

These two points of view are represented by Wright (30) on the one hand, who described them as arising from "bone marrow plasma cells," and by MacCallum (31) on the other, who was satisfied as to the myeloblastic origin of his case even before the oxidase reaction came into use.

The question whether or not Wright and MacCallum were discussing different sorts of tumors is settled by Christian, who studied a series including both Wright's and MacCallum's cases and concluded that they were of the same type. Indeed from a perusal of the typical cases reported in the literature, such as those of MacCallum, Christian, Wright, Vance, and Symmers, and even the older cases of Rustizky, Zahn (32), and Wieland, there can be little doubt that the multiple myelomata represent a distinct tumor type possessing distinctive histological features and differing from sarcomata in general and from the leucemic group. There are no greater differences in cell type in members of individual cases than between any other well defined tumor group. This idea has been especially developed by Christian, who showed that there were not sufficient differences between Wright's and MacCallum's cases to warrant placing them in separate groups, in spite of the fact that the opinion of these two workers differed as to the resemblance of the cells to plasma cells on the one hand and myeloblasts on the other. Christian leaned toward the view that they resemble plasma cells more than myelocytes.

The oxidase reaction has added to our methods for the investigation of these tumors in that it may be applied now to each new case encountered. It must be clearly kept in mind, as Symmers pointed out, that a negative reaction does not prove that the tumor is not of myeloblastic origin. More than this, the author does not find proof in the literature that other cells of the marrow than the myelocyte series *may* not act positively to the oxidase reaction. It must be remembered that the lymphocyte series have been found to react negatively and the more differentiated members of the myelocyte series to act positively under normal conditions; and that while it is logical on these grounds to resort to this technique for deciding the origin of a circulating blood cell, it is quite another and unjustified leap of logic to assume that in the bone-marrow no cells but the myelocyte series give the peroxidase reaction even under pathological conditions. The point is that while the latter assumption may be true the question has not been studied.

In other words the peroxidase reaction applied to myelomata is productive of interesting information, but until we have more definite knowledge of bone-marrow histology nothing is actually settled by applying this reaction to a particular case. Furthermore there are other important general considerations which point toward a solution without the help of the oxidase reaction. To the writer's mind a very important point of view is that of Mallory (33), that these tumors do not consist of cells of the myelocyte series because they do not differentiate like them.

Since Wright claimed a "bone-marrow plasma cell" origin for myelomata there have been many reports either endeavoring to prove or taking it for granted that the tumor arises from the myeloblast series. The objection that has been raised to Wright's view has been that the bone-marrow plasma cell is not a clearly defined entity. As Ewing (34) says, the histological structure of some of the cases so closely resembles plasma cells as to suggest that those who deny their existence have not encountered these cases. The oxidase reaction has revealed a few cases which have reacted positively and which, if we accept this reaction as positive proof of a myeloblastic origin, might be interpreted as myeloblastomata. Forman and Warren (35) report such a case and also Beck and McCleary (36). Both of Symmers' cases were negative as were also those of Pepper and Pearce, Bombard (37), and Vance along with three included in this report.

Aside from the occasional positive peroxidase reaction, the dictum that myeloma multiplex arises from the myeloblast series rests entirely upon an effort to show that the tumor cells resemble myeloblasts morphologically.

This is altogether insufficient evidence considering the fact that its champions object to assigning the tumors a plasma cell origin, although admitting a similar resemblance morphologically. If a resemblance to plasma cells is not a sufficient proof of plasma cell relationships, then a myeloblastic resemblance does not prove myeloblast relationships. Taking the literature as a whole we find both ideas with ample supporters. Thus Von Verebely (38), Thomas (39), Vance, Wright, Christian, Hoffmann (40), Aschoff (41), Pepper and Pearce, and Ewing recognize plasma cell types, while MacLeod (42) and Zininger (43) note a resemblance to plasma cells. Menne (44), MacCallum, Abrikossoff, Weber (45) (Muir), Ribbert (46), Scheele and Herxheimer (47), Sternberg, Jores (48), Symmers, Forman and Warren, Beck and McCleary, believe in the myeloblastic origin.

The question cannot be settled on morphological grounds because of the limitations of the method.

There are several difficulties in the way of considering myelomata as myeloblastic tumors.

In the first place, if the cells of myelomata are myeloblasts or premyeloblasts and belong to the myelocyte series, why are these tumors not chloromata and why do we not find transition stages in structure between myelomata and chloromata? The fact is that myeloma cases never become chloromatous and never become associated with true leucemias, as do all chloromas sooner or later. Furthermore, myeloma cases do not exhibit the changes in the spleen and lymph-nodes that chloroma cases do. It might be argued that the myeloma cell is a less differentiated member of the hemopoietic group and therefore does not possess the highly differential morphological characters of the chloroma cell. Against this is the fact that the chloroma is more malignant than the myeloma, both as regards rapidity of growth, metastasis, and a rapidly fatal issue; which does not argue well for the chloroma possessing a more highly differentiated cell.

In short, chloromata belong to the myeloblast series, are rapidly fatal, and always are, or become, associated with leucemias; myelomata do not differentiate like myelocytes and never become leucemic. Chloromata metastasize widely and are associated with characteristic leucemic marrow and with lymph-node and splenic changes; myelomata do none of these things.

If it were not for the definitely myelocytic or lymphocytic character of chloromata and the definite leucemic nature of these tumors, the possession of a green color alone would not be sufficient to separate them from the myelomata. *To the writer's mind, this failure of myelomata to differentiate like myeloblasts, and the absence of a relation between myelomata and leucemic states are obstacles to accepting myelomata as myeloblastic tumors, no matter how strong morphological evidence based on cytological comparisons may appear to be.*

One most distinctive character possessed by the myeloma is its ability to erode and destroy bone without a reaction being set up. This is striking throughout the literature if we disregard a few isolated cases like the atypical one of Hammers (49), which was was probably not a myeloma. The bone absorption goes on steadily and extensively without attempt at repair, and is certainly a specific property of the tumor cell. Neither normal nor neoplastic cells of the myeloblastic series have this property and the chloromata, although much more malignant than the myelomata, possess very little bone destroying power and the ordinary leucemias scarcely any. The albumosuria is probably associated in some way with the bone destroying power since other destructive bone lesions such as metastatic carcinomatosis sometimes show it and the chloromata and other forms of leucemia only rarely. So far this is in support of the general view Ewing expresses regarding the different identity of typical myelomata and tumors of the leucemic (myeloblastic) group.

A point of view hitherto undiscussed in the literature is that perhaps myelomata belong to that series of reticulum cells whose function it is to absorb bone and regulate bone formation. The statements in the literature regarding bone absorption are unsatisfactory. Two sorts of cells having to do with bone formation have been described, osteoblasts and osteoclasts. By some these have been considered as the same and by others as representing distinct types. The classical cell to which osteoclastic function has been ascribed is a multinucleate giant cell of the foreign-body type. These cells are numerous in areas where large amounts of bone are to be removed such as in healing fractures, but it is remarkable how scarce they are in many sorts of bone formation where a very evident bone resorption is going on in the lacunae.

There has never been sufficient proof that the multinucleated giant cell, which since the work of Kölliker has been assumed to be the active agent in bone absorption, has in fact this function. A very casual survey of sections of actively growing bone, showing evident resorption around the marrow spaces, makes it evident that much bone absorption must go on without the intervention of the classical osteoclast.

This has been recognized by many authors. Arey (50) in a recent important article gives the literature and shows definitely that many workers have been of the opinion that classical osteoclasts are not important in ordinary bone resorption. He concludes that "Only indirect and insufficient evidence points to the osteoclasts as the active specific agents of bone resorption. That they are merely degenerating, fused osteoblasts accords better with known facts." This leaves us with the alternative that the so-called osteoblast must in some way regulate bone absorption as well as bone deposition. This view is favored by

Arey, who believes that bone absorption is brought about as Wells thought by CO_2, although he recognizes that it is "difficult to imagine the mechanism of the localization of carbon dioxide (or the stronger lactic acid) in sufficient concentrations to effect the selective erosion of small areas or to account for the frequent directional polarization of the resorptive wave."

Studying smears and teased preparations from myelomata, the author has been very much impressed by the resemblance which the cells from the tumors bore to the osteoblasts(?) which lie free in the connective tissue of the marrow and especially line the lacunae of rapidly growing bone. This is exemplified by figure 1, which is a photograph of a group of osteoblasts on the tip of a young bone trabecula in an area from the marrow of a rapidly growing ungual osteoma. The basophilic protoplasm, the eccentric nucleus of the vesicular type with peripherally arranged chromatin and central nucleolus are especially striking.

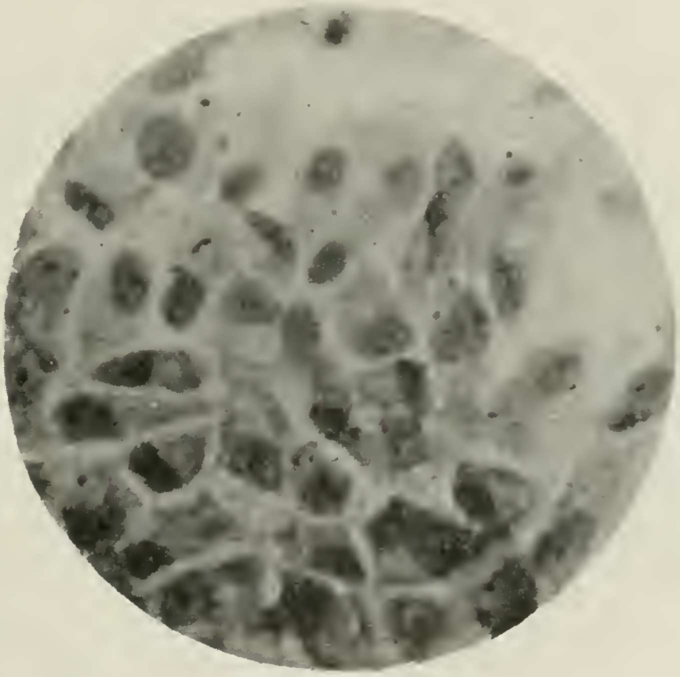

FIG. 1. OSTEOBLASTS IN AN OSTEOMA

It is easy by measurements, staining reaction, and description of cytoplasmic and nuclear structure to show the similar appearance of the "osteoblast" and myeloma cell, but to use this method to establish the identity of the two cells is unjustifiable here as in the case of those who on similar grounds claim to show that myeloma cells are myeloblasts.

The important property of bone absorption which the myeloma cell so prominently possesses, accords well with an inferred relationship to cells of the osteoblastic series. On the grounds of the preservation of this function, we might infer a relative lack of anaplasia and consequent lack of malignancy which again accords with the facts. Only one difficulty arises. If these cells are osteoblastic in their relationships, why do we always find these tumors actively resorbing bone, but never building it?

Either of two assumptions might be favored. First that the cells of the myeloma differentiate in this special direction, and by this mode of differentiation bone absorption is made a hyperfunction just as the production of colloid material by some tumors arising from cells which normally produce it becomes an essential characteristic of their heteroplasia. This requires the conception of a constantly persistent "resorptive wave" to use the euphonic but somewhat vague term of Arey.

The second alternative we might use is to question the fundamental correctness of the idea that the so-called osteoblast is really a bone formative cell and suggest that it is always resorptive in function. This assumption is against the present conception of osteogenesis.

The clinical records of the three following cases have been left out for the reason that they contain no details bearing on the present study.

Case 1. *Autopsy one hour after death.*—The body is that of a very much emaciated man 37 years of age. A globular mass mentioned and described in the clinical history is found involving the left shoulder and clavicle. The seventh rib on the left is fractured in several places and is soft, easily broken, and paper-like throughout. Fractures can also be palpated in the fourth and fifth ribs on both sides.

On section the muscles are dark, dry, and small; the panniculus is absent. Superficial examination of the abdomen reveals the viscera in proper position and no fluid or exudate in the abdominal cavity. On removing the sternum we find it to be thicker than normal and soft and friable, so that large soft portions can be cut out with the knife. By making a mesial section and dividing the sternum in two portions the bone cortex is found to be a mere shell, largely decalcified, and the marrow to occupy almost the entire thickness of the sternum (about 4 cm.). The marrow is mottled pink in color and resembles the cut surface of a lymph-node. There are small hemorrhages and islands of bone fragments. Scarcely any areas of normal red marrow remain.

The ribs are thin-shelled, nearly decalcified, and there are nodules of tumor bulging and breaking through the bony cortex. Elsewhere the medulla of the ribs consists of dry, empty spaces. The right and left lungs are both adherent at the apex and present a low grade, nearly healed tuberculosis. There is anthracosis of the bronchial lymph-nodes.

The heart is of normal size. On section the muscle is deep brown. The aorta presents a moderate nodular sclerosis, evidenced by raised yellowish plaques. In the muscle of the ear of the right auricle, beneath the epicardium, is found a pinkish white plaque similar in appearance to the tumors found in the ribs and sternum. This plaque is about the size of a pea. The heart is otherwise normal.

There is no evidence of thymic remains and the thyroid is of the usual size and on section is found the usual colloid appearance without adenomata.

The gastro-intestinal tract including the liver and pancreas was examined in detail without any important finding. The liver is browner than normal, and appears slightly atrophic (brown atrophy).

The abdominal aorta is moderately sclerotic like the thoracic portion.

The spleen is of the usual size and consistency, and on section seems normal in consistency and appearance.

Both kidneys present numerous white nodules in the cortex. These vary in size from a pin-head to a hickory nut, and have a pinkish white color and lymphoid appearance. They are identical with the tumors found in the ribs and the sternum.

The prostate and bladder are found to be without unusual appearance.

The vertebrae can be freely incised with the autopsy knife. They have almost entirely lost their lime salts and are replaced by the lymphoid-like soft material heretofore described.

Microscopical findings.—The tumor nodules (fig. 2).

The sections of the tumor taken from all the different bones present the same picture. The structure is purely histioid consisting of a uniform mass of closely packed cells with a very delicate reticulum. The blood vessels are without walls, and are in the form of endothelium-lined spaces running through the tumor. All fields present the same picture. There are no areas of degeneration, necrosis, or fibrosis.

At first glance the cell type seems remarkably uniform. The cells are irregularly oval and fairly uniform in size with most of the nuclei eccentrically placed. The protoplasm is slightly basophilic and stains like the protoplasm of plasma cells, and the margins of the cells when not pressed on all sides by other cells tend to be somewhat ragged. On closer analysis the individual cells are found to vary from one another to a considerable degree, but no more widely than those of any tumor which is rapidly growing. The cells vary from an oval shape like that of the plasma cell, to an irregularly round or polygonal outline in those areas where they are pressed upon by other cells. They vary in size from 5.8 to 8 microns in the short diameter, and from 7.7 to 13 microns in the long. The nuclei are more variable in size than the cells themselves. Some of them are small and pyknotic and take up only one end of the cell and others occupy nearly all the cell body. They vary in size from 5.8 x 9.1 microns to round pyknotic forms measuring 3.6 microns. In some of the cells the nuclei have divided without cell division following, so that cells resembling bone marrow megakaryocytes are produced.

Though they vary somewhat in size and shape, the great majority of the nuclei are vesicular in character with definite nuclear membranes which take the stain deeply. The chromatin is rather small in amount, giving the nucleus the appearance of a clear granular vesicle; it is for the most part murally arranged, clinging to the inner side of the nuclear membrane with delicate spiderweb lines running centrally to the nucleolus, which is usually prominent, and varies in size from a tiny chromatin point to a mass measuring about 1.8 microns in diameter. Occasionally there appear to be two nucleoli.

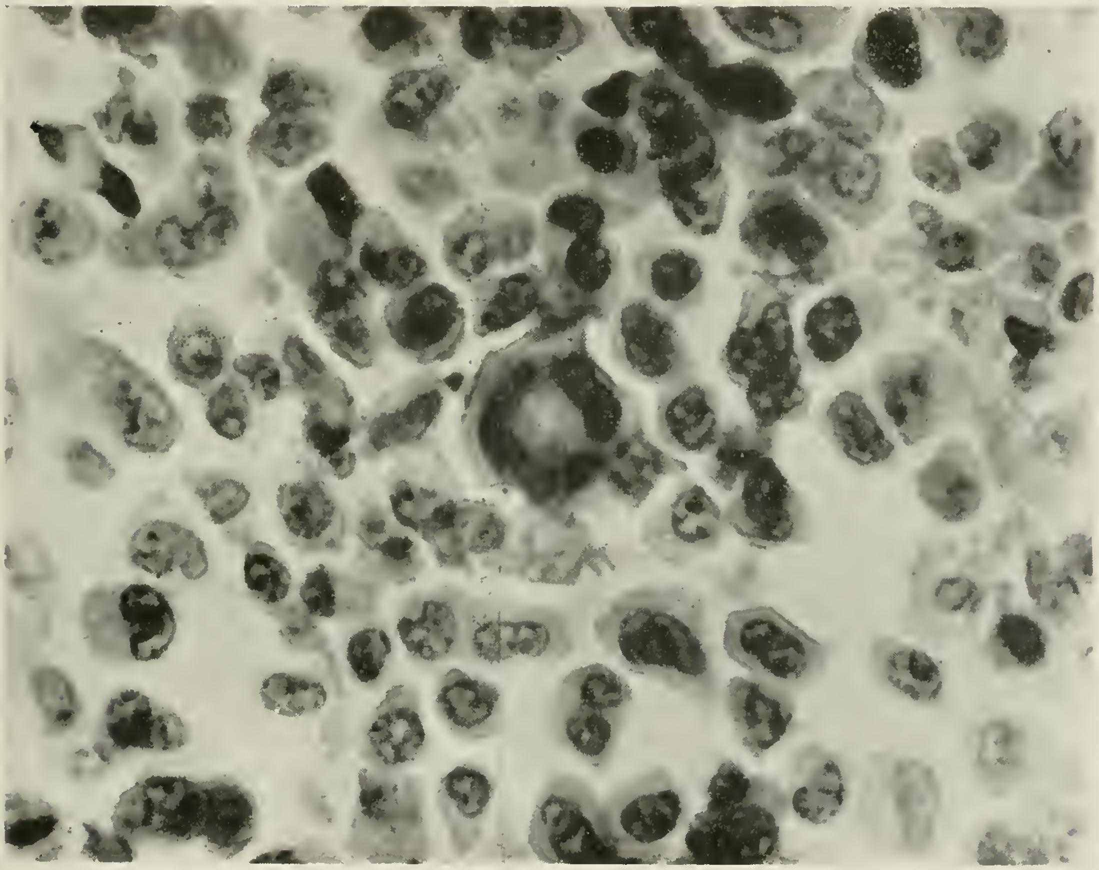

FIG. 2. CELLS OF MYELOMA, CASE I

The sections of tumor taken from the body of the vertebrae show the contiguous muscle to be diffusely invaded. In places bony trabeculae are still present, but undergoing absorption. There are no osteoclasts, but the tumor cells are present in the lacunae and canaliculae and appear to be performing the osteoclastic function. There is no effort at bone formation in any of the sections.

The peroxidase reaction applied by both methods, to smears and frozen sections, was negative.

Kidney.—There is no diffuse infiltration of the kidney parenchyma with the tumor, but there are discrete nodules beneath the capsule about the size of a pea. These nodules have the same histological characters as the primary tumors. Remains of kidney tubules are scattered through it, and glomeruli with their tufts containing many fatty droplets. The uninvolved portion of the kidney shows many areas where the tubular epithelium is completely calcified but no other important changes. An occasional thrombosed glomerular tuft with the capsule filled with wandering cells and an occasional hyaline glomerulus appears, but there are no diffuse changes which could be interpreted as a definite nephropathic condition.

Lung.—There is an advanced emphysema, with the usual accompanying histological changes and a diffuse anthracosis. There are a few heart failure cells. Some of the branches of the pulmonary artery show quite marked proliferation of the intima. The lung tissue as a whole is much more fibrous than is usual in even the more advanced types of emphysema.

The liver presents no important changes. The cells around the central vein contain a great deal of light brown pigment and there is some pressure atrophy of the liver cords in this region with many fat droplets in the cells (early nutmeg liver).

Gastro-intestinal tract negative.

In the heart muscle there is no change except many fine fatty droplets in muscle cells, and increased brown pigment at the poles of the nuclei.

Thyroid.—Moderate enlargement of the follicles. Apparent colloid retention.

Spleen.—The pulp is especially prominent. The Malpighian bodies are few in number and atrophic. The pulp appears remarkably cellular and there are wide sinuses running through it. There is no increase of connective tissue in the spleen pulp.

Bone-marrow.—In areas not grossly involved by tumor growth the normal marrow elements are replaced in part by tumor cells, interspersed in large numbers.

Pathological Diagnosis.—Multiple myeloma of the bones, metastasis to the kidneys and muscle of right auricle. Metastatic calcification of the kidney tubules.

CASE 2. *Autopsy.*—Body is that of a man 35 or 40 years of age. Well formed, distinctly emaciated. Faint icteric tint to the sclera. Moderate purplish hypostasis on the back.

Palpation of the bony cage of the thorax shows many softened crepitating areas on both sides. The left femur just above the knee is fractured when the body is moved from the stretcher to the morgue table. There are no other external abnormalities of importance.

On section the tissue is dry, the muscles dark red. No gas or fluid escapes from the abdominal cavity. Superficial inspection of the abdomen shows no gross abnormality. There is a moderate pseudo-melanosis of the intestines. The fifth rib is softened at the costo-cartilagenous junction, crepitates, and feels irregular on palpation.

Dissecting the tissue away from this region, we find an opalescent bluish mass the size of a hickory nut, and from here to the fourth rib at the costo-cartilagenous junction the tumor mass extends. The tumor tissue found on the fourth and fifth ribs replacing the bony cortex, resembles lymphoid tissue in general. On taking off the lateral wall of the chest with rib shears we find the ribs involved throughout their length by nodules of lymphoid-like tissue, which shine through the pleura presenting a linear nodular appearance along each rib. The ribs are extremely fragile, containing very little lime salts. The tumor mass, primary in the ribs, has completely replaced the rib marrow, everywhere, and in certain places has broken through the cortex and appears as an external nodule.

Removing the sternum with the attached costal cartilages, we find it very flexible, containing very little bone, and much thickened at the lower half. The sternum was split longitudinally with the autopsy knife. Scarcely any lime salts were present.

The marrow has been replaced by a lymphoid mass. The whole costal cage is replaced by lymphoid-like nodular tissue.

The apex of the heart is at the fourth interspace, apparently 1 cm. outside the nipple line. The right heart extends beyond the sternum. Both lungs are free and have collapsed about the hilus. The heart has a small soldier's spot on the right ventricle. The left ventricle is empty. The wall of the left ventricle is normal in thickness and color. The mitral valves admit two fingers. The flaps are normal. The right ventricle is empty. The tricuspid is relatively dilated. The right auricle contains a large post-mortem clot. The pulmonary artery presents no abnormality. The aorta shows a fine nodular type of sclerosis, not particularly advanced. The left coronary mouth is greatly dilated. There are patches of atheroma along the left coronary and also along branches of the right coronary. No gross evidence of syphilis is present in the heart. There is a patch of old adhesions on the posterior surface of the left lung near the vertebrae. The adhesion seems continuous with the tumor in the rib. The tumor has slightly involved the pleura at this point. The lungs are small and dark in color, and on section fairly normal. Mediastinal lymph-nodes show no involvement. There is a purulent discharge from the bronchi. The right lung is small with a few subpleural nodules in the upper lobe. These nodules are calcified, the size of a pea, probably a healed tuberculosis. The right lung is otherwise negative.

The thoracic aorta is normal except for a slight nodular sclerosis.

The spleen is normal in size. There is considerable post-mortem autolysis and on section a distinct hyperplasia of the lymphoid tissue. The left suprarenal is normal in size, and on section shows no changes.

The left ureter is not dilated. The left kidney is small and has a scanty fatty capsule. On section the cortex is pale and swollen. Medullary rays are not well marked. The capsule strips easily.

The right adrenal is normal in size, and on section negative. The right kidney is about normal in size, and on section presents the same appearances as the left.

The mesenteric lymph-nodes are not enlarged. The intestines contain many fecal masses, but are otherwise negative.

Pancreas presents no abnormal gross changes.

There is a moderate purulent cystitis.

The prostate is normal in size.

The liver is of normal size and on section presents a slightly browner appearance than normal.

The gall bladder is distended, but contains no stones.

Pathological diagnosis.—Multiple myeloma of the bones, involving nearly all the bones of the body.

The *x*-ray report of a mass in the mediastinum was based upon an appearance due to the wide infiltration of the vertebrae in the thoracic region by the tumor. On either side of the vertebrae in the upper thorax the infiltration extends outward along the transverse processes and the proximal portion of the ribs in such a manner as to produce a large pyramidal-shaped *x*-ray shadow, which might very well be mistaken for a mediastinal growth.

The following is a summary of the histological changes.

The heart, lung, pancreas, bladder and adrenal present no changes of importance.

The spleen shows a distinct hypertrophy of the lymphoid follicles. There are no important changes in the pulp. No cells resembling tumor cells are found in the pulp. There are a few miliary tubercles in a moderate stage of fibrosis.

There is no increase in connective tissue in the kidney. Glomeruli appear normal. The cells of the tubules are granular from postmortem change. Throughout the cortex are many tubules which have been replaced by lime salts.

Pieces were taken from the tumor nodules of almost all the bones in the body, for microscopical examination.

The picture presented is uniform throughout (fig. 3). The tumor consists of a uniform histioid structure with a uniform type cell arranged on a delicate reticulum, with an intimate relation between the tumor cells and the blood-vessels. The blood-vessels have no proper wall, but pursue their course through the tumor lined by a simple layer of endothelial cells. The tumor cells have the usual resemblance to plasma cells. They are of the same size and variation as in the previous case. In general they have eccentrically placed nuclei, basophilic protoplasm, with large vesicular nucleus showing the characteristic mural arrangement of chromatin with fine threads projecting inward toward the nucleolus. Although the ribs, vertebrae, sternum, long bones, and bones of the pelvis were involved in this case, there were no metastases to other organs as in the previous case. The metastatic calcification of the kidney was quite as striking as in case 1. There were no other pathological changes of importance found by microscopical examination of the sections.

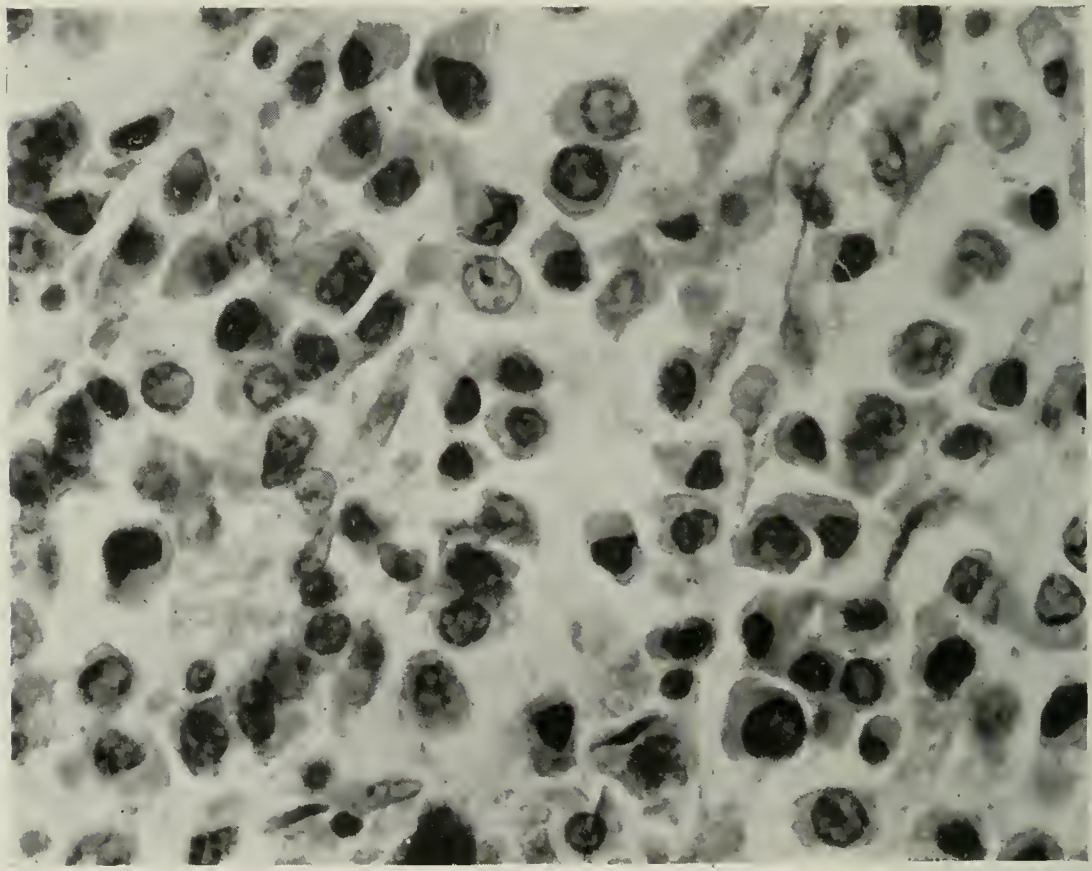

FIG. 3. CELLS OF MYELOMA, CASE II

The peroxidase reaction applied to smears of the tumor as well as to frozen sections of tissue fixed in formalin were negative by both methods.

Case 3. *Autopsy findings.*—The body is that of an adult male, about 40 years of age, 5 feet 11 inches tall. Greatly emaciated. The bony landmarks are normal throughout.

About 10 cm. to the right of the mid-sternal line on the third rib is a subcutaneous lump the size of a hen's egg and a smaller lump in the same region in the first rib under the clavicle. These lumps are connected with the rib and continuous with it.

There is some enlargement of the left testis, which is soft and elastic.

The abdomen is slightly distended. On section the panniculus is found practically absent. Tissues are moist and the muscles dark red. Superficial inspection of the abdomen shows a small amount of fluid in the abdominal cavity, but the gross relation of the organs is normal.

On stripping back the muscles from the thoracic cage we see the nodules above described developing from the ribs. The bony structure of the ribs is soft and friable. The mass is enlarged, has broken through the bony cortex of the ribs, and on section one obtains a thick semi-gelatinous substance, somewhat resembling pus.

The right lung is adherent in the upper portion and laterally by well organized fibrous tissue.

The left lung is free, but throughout there is considerable hypostatic congestion.

The right lung shows marked congestion, some edema. There are no solid areas of pneumonia and the bronchial lymph-nodes are not enlarged. There is considerable anthracosis. The left lung is less congested than the right. Both lungs crepitated throughout.

The heart lies free in the pericardial sac. There are no adhesions. There is a small amount of free fluid in the pericardial cavity. The mitral valves admit the tips of two fingers. There is marked sclerosis of the mitral cusps. The tricuspid valves are negative. The right orifice is empty, the left orifice admits two fingers, but there is no evidence of sclerosis. There is considerable edema of the walls of the coronary arteries. The aortic semilunars are slightly sclerotic. The first portion of the aorta is somewhat dilated. The intima has a slight reddish discoloration, from a post-mortem imbibition of hemoglobin. There are no other changes of importance in the thorax.

In the abdomen there is about 200 to 500 cc. of clear fluid. The relation of the abdominal viscera is not disturbed. The liver is slightly enlarged, extending about two finger-breadths below the costal margin. It is smooth, the capsule somewhat thickened. On section the liver bleeds easily, and the centers of the lobules are dark red in color. The spleen is normal in size; the capsule somewhat thickened. On section there are no important gross changes. On removing the intestines we find the pyloric end of the stomach adherent by a mass of soft friable tissue. The mass involves the wall and the pyloric ring, and on section has the same general character as the mass found in the rib. This mass is continuous with the posterior wall of the abdominal cavity where it extends into the 8th and 9th dorsal vertebrae. These vertebrae are completely destroyed by the tumor, and the mass in the stomach and the abdomen is evidently a direct extension from this point.

Inspection of the vertebral bodies as a whole shows practically the whole spine involved in the process, with some areas of fairly normal vertebral tissue between the nodular masses of tumor substance.

The retroperitoneal lymph-nodes are somewhat enlarged.

The pancreas is soft, slightly displaced by the mass adherent to the pylorus.

The kidneys are somewhat congested, but show no other gross changes.

The prostate is slightly enlarged.

There is a large right-sided hydrocele of the testis, with some atrophy of the organ. The left testicle is slightly enlarged, but shows no gross changes.

Pathological diagnosis.—Multiple myeloma of the bones. Direct extension to the stomach. Hydrocele of the right testis.

Microscopical findings.—The lung shows a distinct emphysema with areas of atelectasis. There is fibrosis of the interalveolar septa, and many heart failure cells in the alveoli. The bronchi are filled with a thin mucous secretion. There is an advanced anthracosis. There is a considerable hyperplasia of both the spleen pulp and the Malpighian bodies. The spleen pulp shows an increase in its cellular content, and under high power many of these cells resemble those of the tumor. In the margins of the Malpighian bodies are also large numbers of large mononuclear cells, identical morphologically with those found in the tumor nodules. There is considerable increase of connective tissue in the pulp. The kidney presents an appearance of postmortem disintegration of the protoplasm of the tubules. No changes are found which can be interpreted as nephropathic.

In sections of the prostate there are found no inflammatory areas or areas of glandular hyperplasia.

There are no changes of note in the heart muscle.

Sections were made from the tumor nodules of the rib of the involved vertebrae, and of the stomach region involved by continuity with the vertebral tumors. The tumor is of a pure histioid character, consisting of round cells arranged on a delicate connective tissue reticulum (fig. 4). There are some larger bands of hyaline connective tissue running through the tumor. The blood-vessels have delicate walls consisting of a few connective tissue strands and lined by a delicate endothelium. The cells in general closely resemble plasma cells. They are somewhat ovoid in shape with an eccentrically placed nucleus and a basophilic protoplasm; their margins tend to be somewhat ragged where the cell is not pressed upon from all sides. The nucleus is vesicular with murally arranged chromatin and fine bands of chromatin running toward the center to a rather large nucleolus. The structure is typical of a myeloma of the so-called plasma celled type.

In none of these three cases were abnormal cells observed in the peripheral blood. The many cases in the literature in which myelocytes in small numbers have appeared along with a terminal polymorphonuclear leucocytosis are explained on the ground that such terminal blood pictures are common with advanced tumors, especially when the bone-marrow is involved. Our cases show the terminal leucocytosis common to late malignancies, as pointed out by Vaughan (53).

The author does not find convincing statements in the literature that "plasma cells" or cells of distinctive type may be found during life in the circulating blood and used for diagnosis. The case of Beck and McCleary is certainly open to question regarding the interpretation of the origin of the cells found at autopsy in the blood-vessels. In the first place these cells had already been interpreted as ordinary blood cells during life, and not until after death was it decided that they represented distinct tumor cells. Further, although the patient is said to have died of acute lobar pneumonia, the white blood count is recorded as 8800. The tumor cells in their case gave the indophenol blue synthesis, but they do not record its result with blood smears.

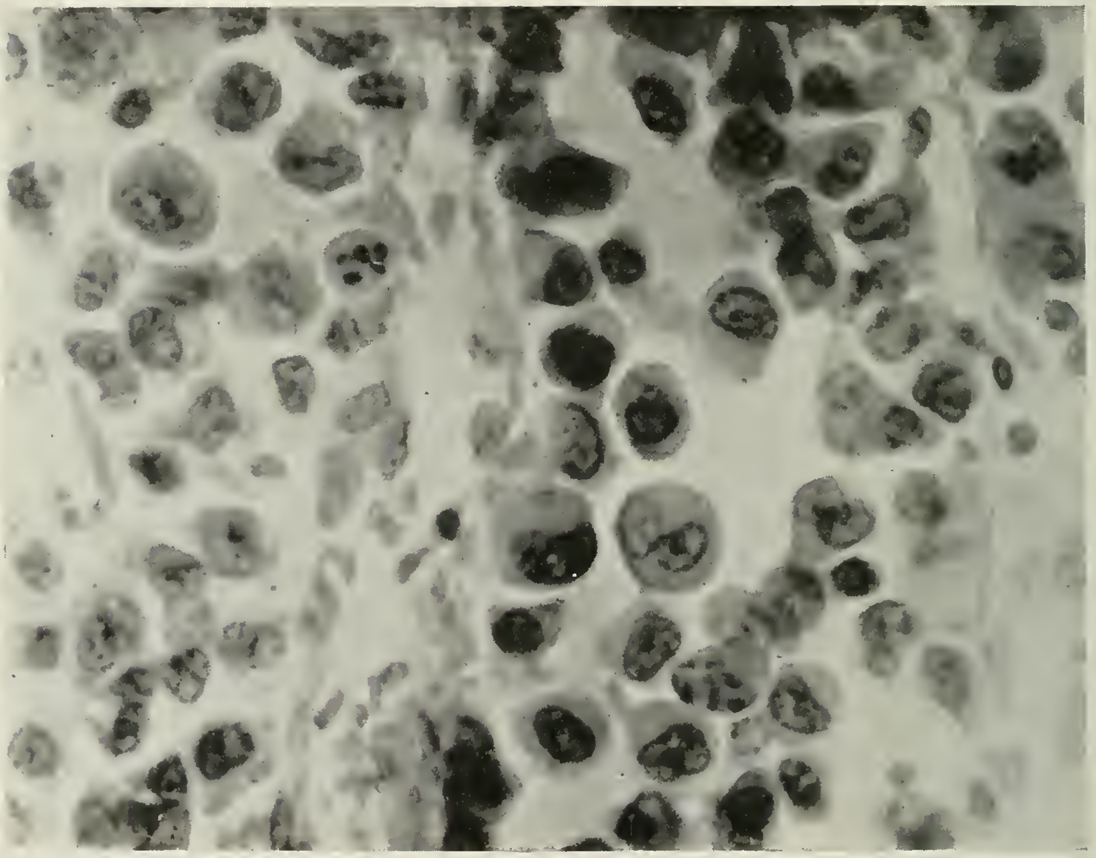

FIG. 4. MYELOMA OF THE "PLASMA-CELL TYPE," CASE III

The finding of abnormal cells in these cases must be interpreted as a result of combined reaction to late malignant disease in general and to non-specific bone marrow involvement in particular.

The peroxidase reaction, both on frozen section and on smears from the tumor surface, was negative by both methods tried. The methods in detail are given below.

THE PEROXIDASE REACTION

Two methods were used on both smears and frozen sections. The technique used by Forman and Warren was applied, which is similar to the one used by Evans (51) except that some steps are omitted.

The frozen sections from formalin-fixed tissue were placed in equal parts of 1 per cent alpha-naphthol in 1 per cent KOH and of 1 per cent dimethylparaphenylendiamin for two minutes. The section was then washed and examined in water on a slide with a coverglass. Smears were fixed in formol-alcohol for two minutes, washed, and the same technique applied.

The second method used was that of Goodpasture (52). The stain is prepared as follows:

Alcohol 95 per cent	100.0 cc.
Sodium nitroprusside	0.05 gm.
Benzidine (C. P.)	0.05 gm.
Basic fuchsin	0.05 gm.
Hydrogen peroxide	0.5 cc.

The sodium nitroprusside is dissolved in as little water as possible and then added to the alcohol; the other ingredients are then included. Goodpasture's solutions retained their activity for several weeks. I have not been able to get the reaction except with freshly prepared solutions.

Smears are allowed to dry and then covered with the reagent for one minute; an equal quantity of water is added and allowed to remain three minutes when the preparation is washed, blotted, dried, and mounted in balsam.

Frozen sections of formalin-fixed tissue are placed in equal parts of the reagent and water for five minutes, washed in water, dehydrated in acetone, cleared in xylol, and mounted in balsam.

The oxidase granules are blue, the nuclei red, and the protoplasm pink.

Blood smears and frozen sections from an inflamed Fallopian tube and appendix were used to control the stain.

In all three cases only a few scattered leucocytes showed the characteristic granules in either sections or smears. From this result we can only conclude that if the cells are of the myelocyte series they are too imperfectly differentiated to give the reaction.

SUMMARY AND CONCLUSION

1. Three cases of non-oxidase reacting myelomata are reported.
2. The histogenesis of these tumors is discussed, and data presented which is interpreted to support the theory that the so-called "plasma cell" type of myelomata is not of myeloblastic origin and has no relation to the leucemic group.
3. It is suggested that the "plasma cell" myelomata spring from a series of cells whose specific function is bone absorption, and that the myeloma cell may be a heteroplastic "osteoblast."
4. The finding of abnormal cell types in the peripheral blood of myeloma cases has not been demonstrated by the published examples to be specific and characteristic of this form of tumor. The myelocytes, "plasma cells," and other abnormal cell types, together with varying degrees of leucocytosis and disturbances of the percentage relationships of the various normal leucocytes, are adequately accounted for by the condition of malignancy accompanied by wide-spread bone-marrow involvement, and is found in non-myelomatous conditions.

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THE INFLUENCE OF CERTAIN DIETS UPON TUMOR SUSCEPTIBILITY AND GROWTH IN ALBINO RATS

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The question of the relationship between neoplastic growth and the food supply of the host has received the attention of a number of investigators. Such studies have not been limited to experimental animals, and a number of writers have suggested a relationship between diet and malignant disease in man. Thus Ross (1) reports that among the natives of the Arctic region cancer is rare. It has been suggested that the rarity of cancer among the natives of Africa, India, the East Indian Islands, China, Japan, and Brazil, is due to their living entirely upon a vegetable diet. However, those who have adopted the European life and eaten a large quantity of meat are not exempt from malignant disease (2–6). On the other hand, Hendley (7), Bentall (8), and Bashford (9) have reported that the occurrence of cancer among the vegetarian Japanese and Indians is as great as among those indulging in a mixed diet.

It may be of interest in conjunction with the present paper to review briefly what dietetic studies have been made on the nature and cause of malignant growth.

Haaland (10) has shown that mice fed upon a diet consisting of hemp-seed, bread, milk, and oats are less resistant to a transplantable Ehrlich mouse sarcoma than those fed upon a less complex diet of bread and oats.

Stahr (11) has reported that a diet of hemp-seed and milk makes mice less susceptible to tumor inoculation than those fed upon bread and water.

Moreschi (12) studied the development of tumor grafts in underfed mice, and found that these growths are very much retarded by such treatment. His results were confirmed by Rous (13).

Sweet, Corson-White, and Saxon (14) showed that the number of takes of a transplantable mouse carcinoma is greatly reduced and the rate of tumor growth is retarded if the hosts are fed upon Osborne-Mendel's artificial diet (15) in which certain amino-acids are lacking. A similar experiment with Flexner-Jobling rat carcinoma showed the same results and they drew the conclusion that tumor cells and normal somatic cells are subject to the same laws of growth (16).

Van Alstyne and Beebe (17) found that if the hosts were fed with non-carbohydrate diet from two to six weeks previous to transplanting tumor tissue, the resistance of the animals to the tumor became greater and the growth of the grafts was retarded as compared with those fed upon a complete diet. They recalled the work of Robertson and Burnett (18) and suggested that the difference in tumor susceptibility in animals fed upon a mixed diet of oats and meat and exclusively milk diet is partly, if not entirely, due to quantity difference in the carbohydrate content of the diets.

Rous (19) made an extensive study of the influence of Sweet's modification of Osborne-Mendel's artificial diet on tumor grafts and on spontaneous tumors. He showed that such diet had no specific influence upon the growth of Flexner-Jobling adenocarcinoma, but had a distinct retarding action upon the metastasizing mouse carcinomata. In studying spontaneous tumors he obtained the following results: first, that underfeeding the tumor-bearing animals with such diet for some days prior to the removal of all except a small portion of tumor delayed recurrences and the growth of tumor grafts; second, changing to the special diet from normal diet after the operation had no marked effect on the number of recurrences or the time required for their appearance, though the growth of grafts was somewhat retarded; and thirdly, that unoperated spontaneous tumors appeared to be unaffected by the most drastic dieting. From these results he concluded that the dietetic influence upon tumor growth may be "attributed solely to the underfeeding and resultant loss of weight, and not to the character of the food" (20).

Funk (21) reported that chickens (Plymouth Rocks) fed upon polished rice a few days prior to inoculation of Rous's chicken sarcoma showed no takes; however, if they were fed upon unpolished rice or polished rice with the addition of a small amount of yeast, the tumors developed. A similar experiment by Levin (22) did not support Funk's observations.

One of the present writers and Rahe (23) found that an artificial diet free from water-soluble vitamines had a retarding action upon the development of tumor grafts, and that the tumors could obtain a supply of necessary vitamines from the tissues of the hosts.

Drummond (24) has studied the influence of the following dietary inadequacies upon tumor and normal tissue growth: (a) low protein content of the diet; (b) nitrogen of the diet supplied in the form of a protein possessing a relatively low nutritive value; (c) absence of certain indispensable amino-acids from the diet; and (d) absence of the equally indispensable accessory growth promoting factors, the "fat-soluble A" and the "water-soluble B." The artificial diet proposed by Osborne and Mendel (25) was used. Drummond's experimental results corroborate the findings of Sweet and associates (16) as to the amino-acids deficiency; of Benedict and Rahe (23) as to the "water-soluble B" deficiency; and of Rous (19) as to the undernourishment of the hosts, and he has lastly stated "that only the most drastic restrictions, involving a very serious loss of weight upon the part of the host, have any retarding influence upon tumor growth."

In the present study we have selected the banana as the basis of our experimental ration. It is the main article of diet of the natives of several tropical countries (Egypt, Brazil, etc.), where the incidence of cancer is low. The nutritive value of the banana has already been studied (26). The fruit is deficient in (a) protein, and (b) certain food accessory factors or so-called *vitamines*.

EXPERIMENTAL

Methods and materials employed

Throughout the present experiments we have used the Flexner-Jobling rat carcinoma which was kindly furnished by Dr. Wood,[1] Director of the Crocker Laboratory of Columbia University. This tumor was in the 60th generation at the time we obtained it, October 22, 1918, and was designated in that institute as $\frac{\text{FRC}}{\text{60D}}$.

The previous history regarding this specific tumor has been given by Flexner, Jobling, and others. The tumor was very malignant in character, having, (a) power of uniform, rapid, and continuous growth; (b) power to metastasize, etc.

The tumors to be used for the successive transplantations were selected among rapidly growing tumors which had not ulcerated. The age of the tumors was about four weeks.

The general routine of the tumor inoculations (27) was as follows: After killing the tumor-bearing animal with ether it was submerged in 0.1 per cent of mercuric chloride or 1 per cent of lysol for about a minute, and then in 95 per cent ethyl alcohol for about the same time. The tumor was removed by sterile instruments and transferred to a sterile Petri dish. A portion of the tumor was fixed in a formaldehyde solution for histological examination. The subcutaneous inoculation of the tumor fragments into new hosts was done by a trochar which was sterilized by alcohol and heat and cooled in sterile water between inoculations. Tumor tissue weighing about 10 mgm. was selected from the non-necrotic area of the tumor and was inoculated into the region of the right axilla. The site of the inoculation, about 5 cm. below the axilla, was sterilized by rubbing with cotton soaked with 95 per cent alcohol. Animals were not anaesthetized for the transplantation.

The weight of each animal was recorded every third or fourth day and the approximate size of its tumor was measured each week by means of calipers, and a drawing was made according to measurement. The final measurement and the weight of the grafted tumor were obtained at autopsy.

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[1] The present writers wish to express their obligation to Dr. Wood, and also to Dr. Itami for his advice and suggestions concerning tumor inoculation.

Tumor inoculation in animals fed upon the special diets and in control animals fed upon a normal diet was performed simultaneously.

The albino rats used were exclusively of our own laboratory breed from the stocks of a New York dealer and of Cornell Medical College. The normal diet consisted of wheat-bread soaked in whole milk, and fresh carrots or cabbage.

The animals, both males and females, selected for the comparative experiments were from 50 to 150 days old at the time of inoculation.

Fresh food was given each morning. The amount of food was not restricted throughout the series of experiments. Fresh tap water was given ad libitum.

The following special diets were employed. The methods of preparation for the individual food substances have been fully discussed in a previous article (28).

TABLE 1

Special banana diets

NAME OF SUBSTANCE	RATION A	RATION B	RATION C	RATION D
	A complete banana diet	A complete banana diet after lactation	Partially deficient in food accessory factors	Deficient in protein and partially deficient in food accessory factors
	per cent	*per cent*	*per cent*	*per cent*
Bananas	83.0	83.5	84.0	100
Casein	16.0	16.0	16.0	0.0
Yeast	0.5	0.5	0.0	0.0
Protein-free milk	0.5	0.0	0.0	0.0

A parabolic growth curve of the Flexner-Jobling rat carcinoma

During the past sixteen months we have made twenty successive transplantations using from 6 to 24 albino rats fed upon normal diet for each series, and have successfully grafted from 70 to 100 per cent of the animals, seldom as low as 50 per cent.

The development of these tumor grafts during the first few days was gradual and then continued very rapidly until the tumors broke down owing to their size and to injuries (Chart I). However, the rate of weight increase of the tumor is highest in the early stage of development, due to its rapid cell division, and falls rapidly until it reaches about the twenty-first day, and then very slowly declines. The curve of growth is smooth (Chart II).

The progressive growth of these tumors was obtained when the diet of the hosts was complete. We see from the data given in table 2 that the materials required for building up these new growths in the earlier days were small; and thus the body weights of the hosts remained unaltered.

TABLE 2

Showing the mean weight and dimensions of the Flexner-Jobling rat carcinoma on seven selected days

AGE IN DAYS	NUMBER OF TUMORS EXAMINED	AVERAGE WEIGHT OF TUMOR	AVERAGE SIZE OF TUMOR	RATE OF INCREASE IN WEIGHT
		gm.	*cmm.*	*per cent*
0	15	0.010	14.8	
7	11	0.113	196	1030
14	8	0.878	1280	677
21	10	3.10	4570	253
28	14	6.29	9040	103
35	17	10.8	16900	72
42	21	18.3	32200	70

We have noticed that the hosts generally remained in good health for nearly 42 days from the day of inoculation even though they slowly lost weight, possibly because the nutritive requirements of the tumor exceeded the food intake of the host and because the activity of the tumor disturbed the metabolism of the host.

Table 2 gives the average weight and dimensions of the tumors exclusive of cystic, local metastatic, and ulcerated tumors, obtained by killing and autopsying animals of a given age.

In Chart I we have shown the parabolic growth curve of the Flexner-Jobling rat carcinomata, the almost identical growth curve of fetuses (29), and also the linear growth of young albino rats during the period of lactation (28).

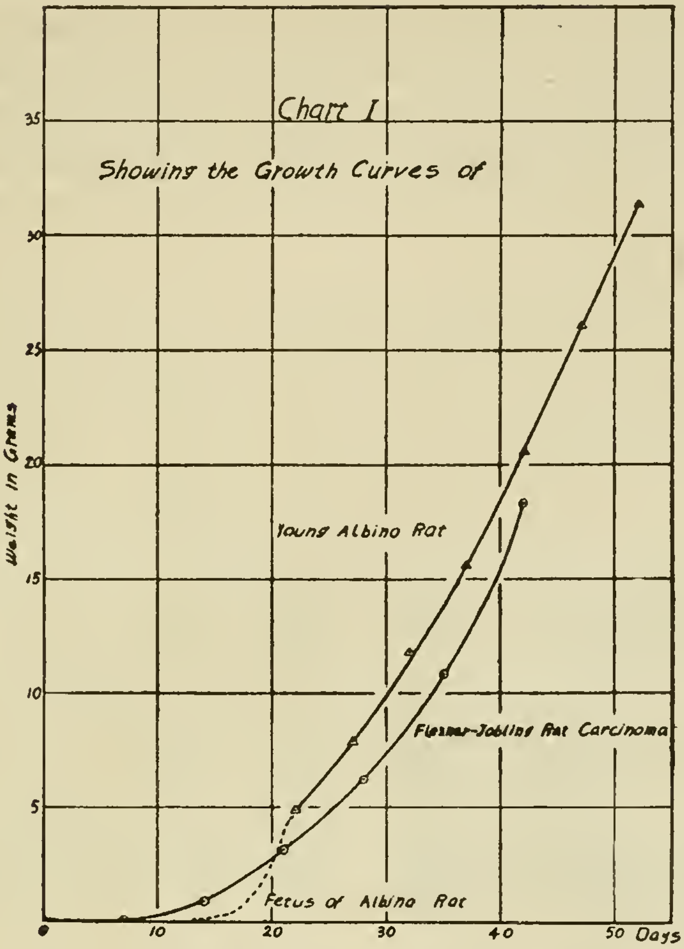


Chart I
Showing the Growth Curves of
Young Albino Rat
Flexner-Jobling Rat Carcinoma
Fetus of Albino Rat
Weight in Grams
35
30
25
20
15
10
5
0
10
20
30
40
50 Days

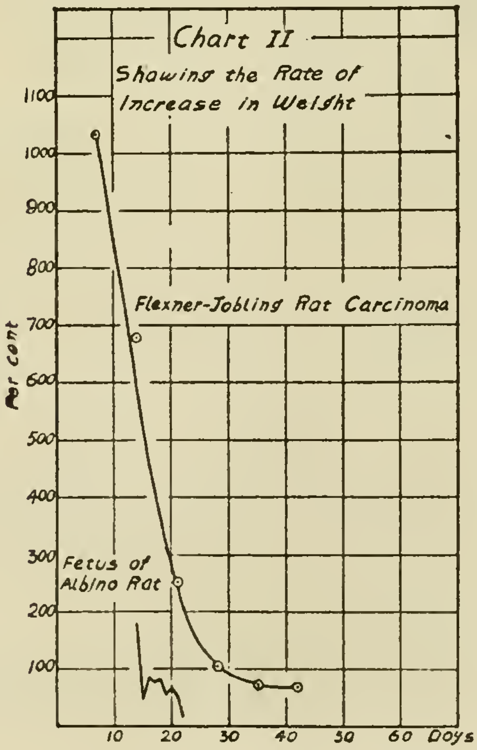
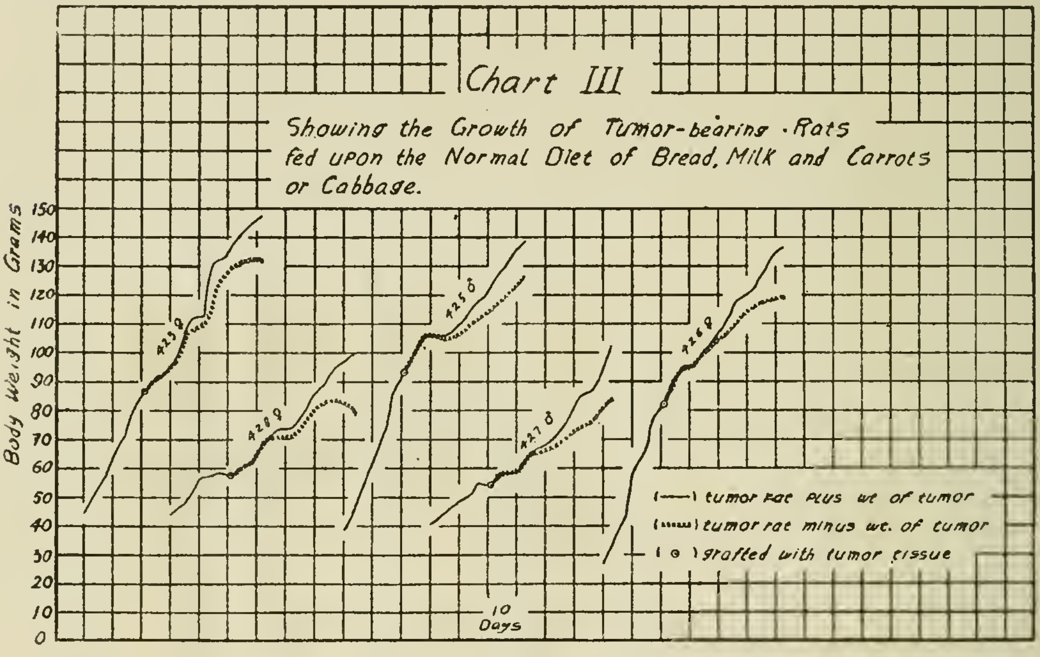


Chart II
Showing the Rate of Increase in Weight
Flexner-Jobling Rat Carcinoma
Fetus of Albino Rat
Per cent
1100
1000
900
800
700
600
500
400
300
200
100
10
20
30
40
50
60 Days

Chart III shows the growth curves of tumor-bearing rats fed upon a normal diet of bread, milk, and carrots or cabbage. The apparent weights of the tumors in each succeeding week except the final were made with the help of diagrams and surface measurements.



The influence of two complete diets, different in nature, upon tumor growth and susceptibility

Experimental results by Osborne and Mendel, McCollum, and others, have shown that for very young growing animals the selection of the protein and food accessory substances is a matter of great importance, and also that the building up of their body tissues requires a relatively much greater amount of the growth promoting accessory factors than the adult animal requires.

It has been shown in a former article by the present writers that a diet consisting of bananas, 83 per cent, casein, 16 per cent, yeast, 0.5 per cent, and protein-free milk, 0.5 per cent is an adequate diet for the growth, maintenance, reproduction, and perfect milk production of the albino rat.

Experiment 1, September 18, 1918.

Eight young rats about two months old were placed on this complete banana diet, seven control animals of a similar age and weight were fed upon the normal diet of bread, milk, and carrots or cabbage. Thirty-four days later tumor fragments were inoculated into these animals under aseptic precautions. The results of this experiment are shown in figures I and II.

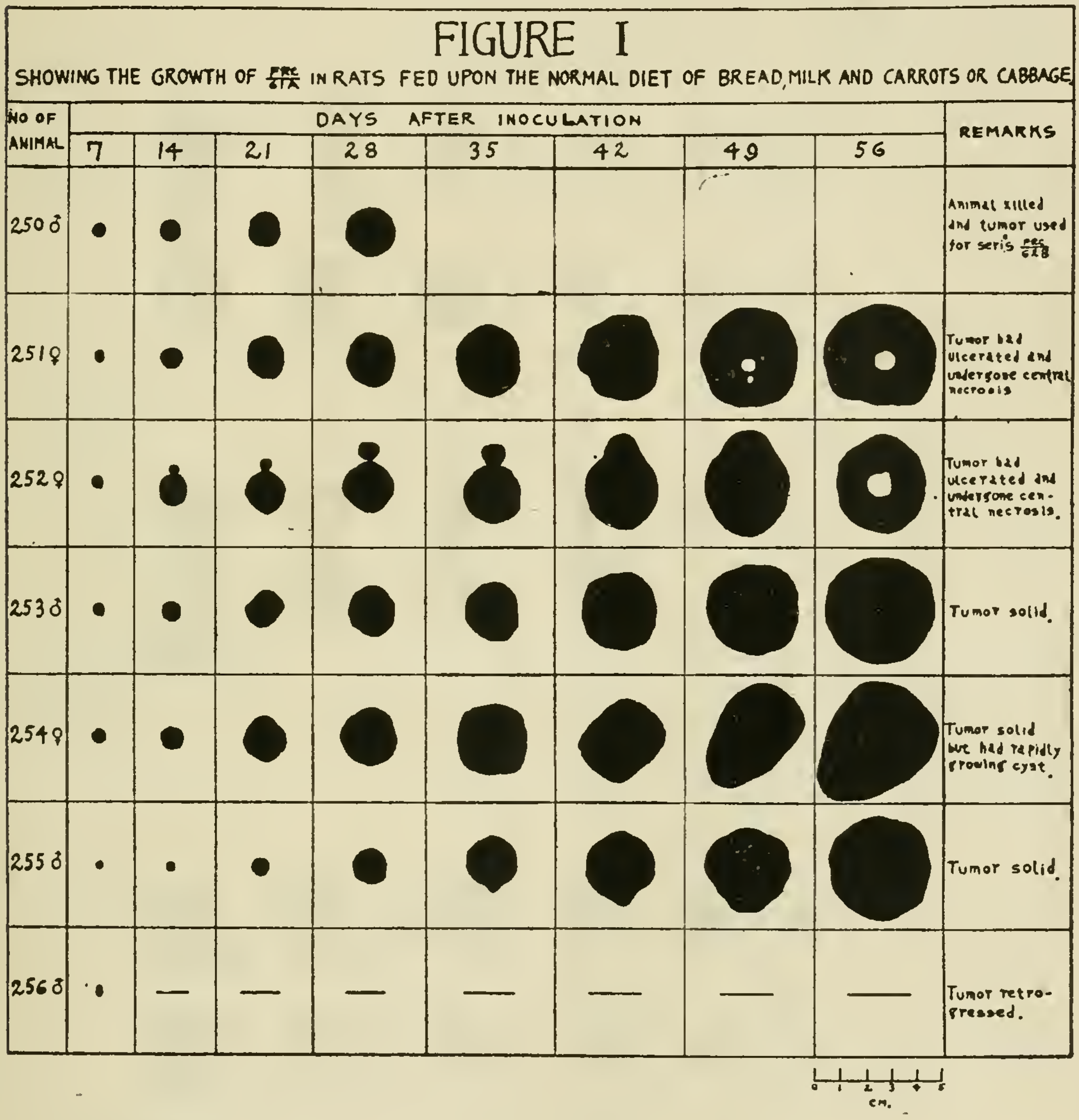


FIGURE I

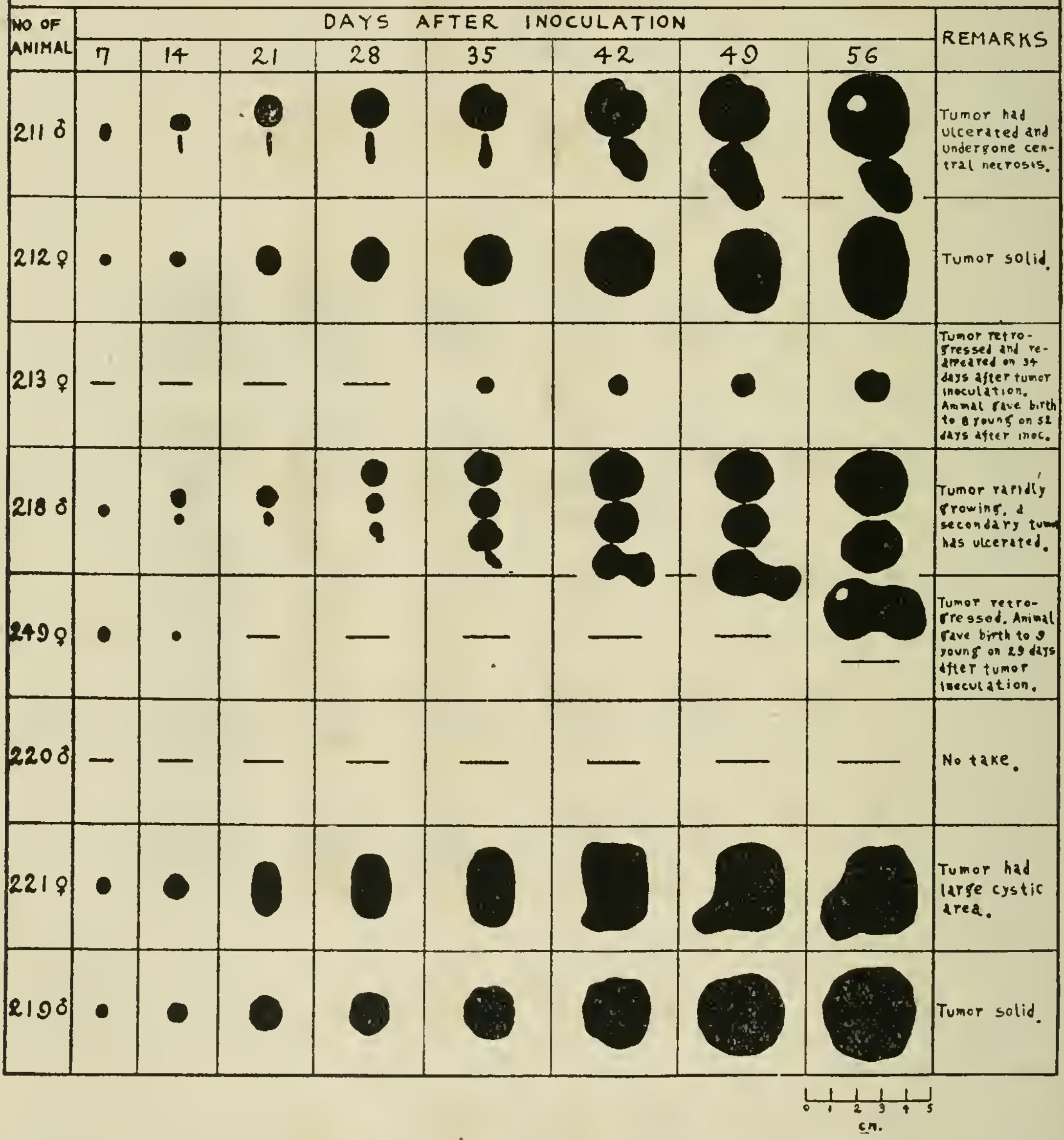
SHOWING THE GROWTH OF $\frac{FRC}{6TA}$ IN RATS FED UPON THE NORMAL DIET OF BREAD, MILK AND CARROTS OR CABBAGE

Experiment 2, November 19, 1918.

In order to eliminate differences in successful takes which might have resulted from the previous diet, we selected ten young rats from two litters of fourteen young which were successfully raised by mothers whose diet consisted of bananas, casein, yeast, and protein-free milk exclusively, and continued feeding with the same diet before and after tumor inoculation. The average weight of the animals at the time of the tumor inoculation was 105 grams. A similar procedure was carried out on

FIGURE II

SHOWING THE GROWTH OF THE $\frac{FRC}{61A}$ IN RATS FED UPON A COMPLETE BANANA DIET OF BANANAS, 83.0; CASEIN, 16; YEAST 0.5; AND PROTEIN-FREE MILK, 0.5 PER CENT.



Tumor had ulcerated and undergone central necrosis.

Tumor solid.

Tumor retrogressed and reappeared on 34 days after tumor inoculation. Animal gave birth to 8 young on 52 days after inoc.

Tumor rapidly growing, a secondary tum has ulcerated.

Tumor retrogressed. Animal gave birth to 9 young on 29 days after tumor inoculation.

No take.

Tumor had large cystic area.

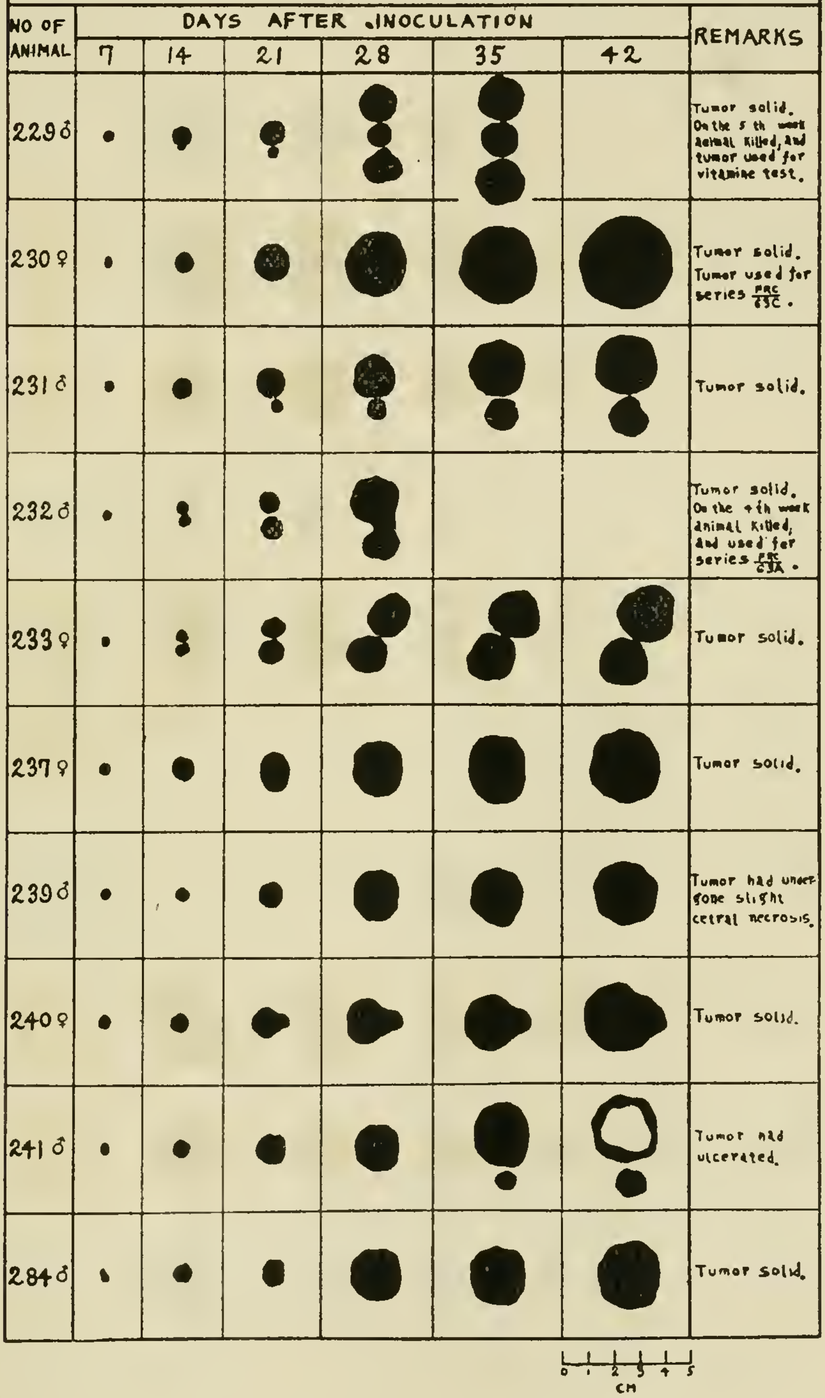
Tumor solid.

those control animals fed on a normal diet. The results of this experiment are shown in figures III and IV.

These two experiments show very clearly that the growth of, and susceptibility to the tumor in rats is not altered by the difference in the *character* of the food given in these experiments.

FIGURE III

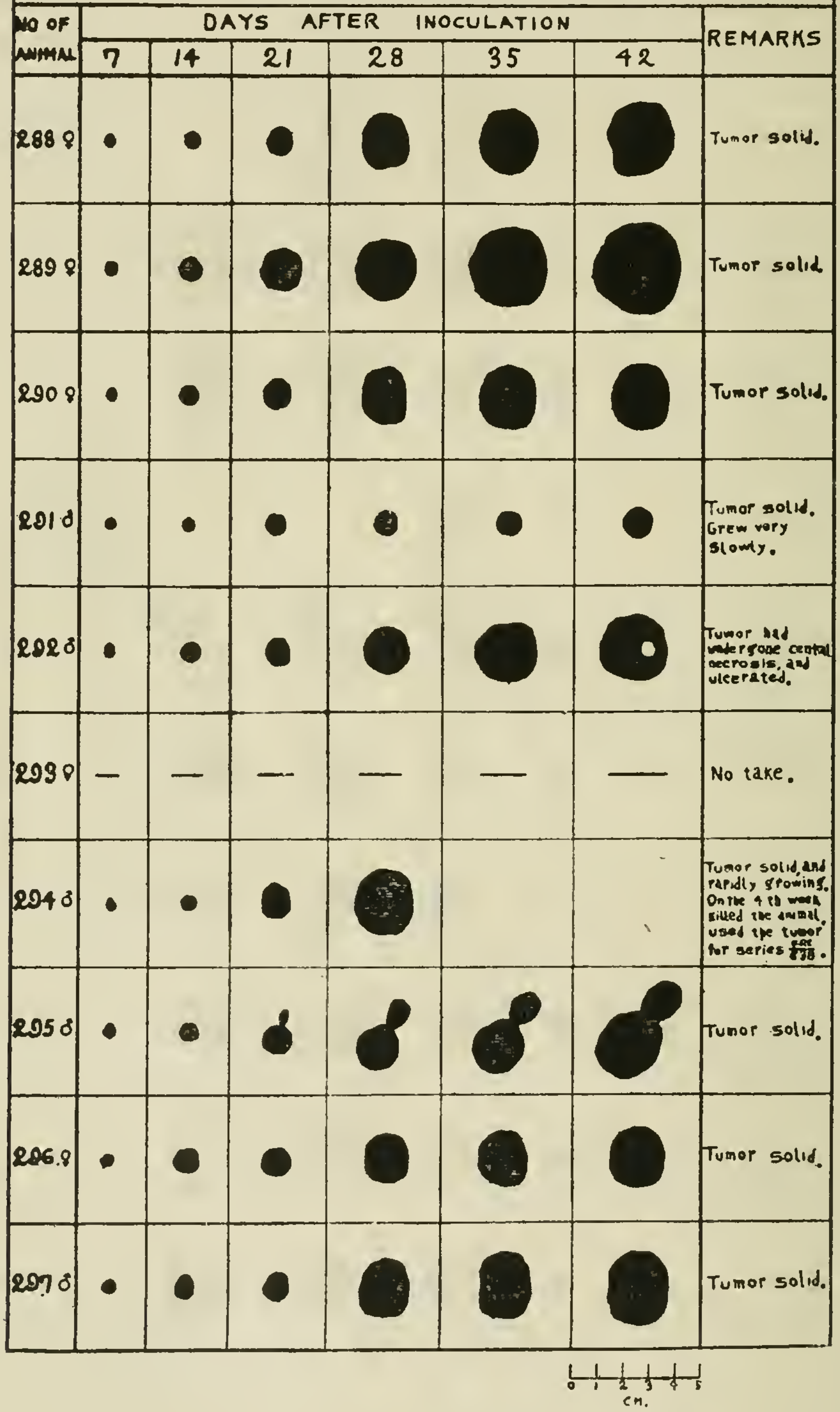
SHOWING THE GROWTH OF THE $\frac{FRC}{62B}$ IN RATS FED UPON THE NORMAL DIET OF BREAD, MILK AND CARROTS OR CABBAGE.



NO OF ANIMAL	REMARKS
229♂	Tumor solid. On the 5 th week animal killed, and tumor used for vitamine test.
230♀	Tumor solid. Tumor used for series $\frac{FRC}{65C}$.
231♂	Tumor solid.
232♂	Tumor solid. On the 4 th week animal killed, and used for series $\frac{FRC}{63A}$.
233♀	Tumor solid.
237♀	Tumor solid.
239♂	Tumor had undergone slight cetral necrosis.
240♀	Tumor solid.
241♂	Tumor had ulcerated.
284♂	Tumor solid.

FIGURE IV

SHOWING THE GROWTH OF THE $\frac{FRS}{228}$ IN SECOND GENERATION RATS WHOSE MOTHERS AS WELL AS THEMSELVES WERE FED UPON A COMPLETE BANANA DIET, WHICH CONSISTED OF BANANAS, 83.0, CASEIN, 16.0, YEAST, 0.5, AND PROTEIN-FREE MILK, 0.5 PERCENT.



We have shown that a mixture of bananas, casein, and yeast provided all the food requirements of young rats for normal growth and reproduction (26). Such a diet is, however, deficient in some substance essential to the production of proper milk by the mother. In the next experiment the effect of this slightly deficient diet upon the growth of tumors was studied.

Experiment 3, April 3, 1919.

Twenty young healthy rats were divided into two groups. Ten of them were placed on a special diet of bananas, 83.5 per cent, casein, 16 per cent, and yeast, 0.5 per cent, while the other ten were fed upon the normal diet. Fourteen days later tumor fragments were inoculated into all these animals under aseptic precautions.

The results of this experiment are shown in figures V and VI.

According to figures V and VI the development of the tumor grafts in the animals fed upon a complete banana diet was somewhat retarded. Most of these tumor-bearing animals showed subnormal growth throughout the experimental period. At the end of the sixth week it was found that the average weight of the tumors was 6.1 grams. On the other hand, the control animals, fed upon bread, milk, and carrots or cabbage, increased rapidly in weight, as we have shown in Chart III. The average weight of the tumors was 16.4 grams, or more than two and a half times that of the specially fed animals.

We may conclude, however, that the retarded growth of the tumors was probably due to the influence of the subnormal growth of the hosts in this particular experiment.

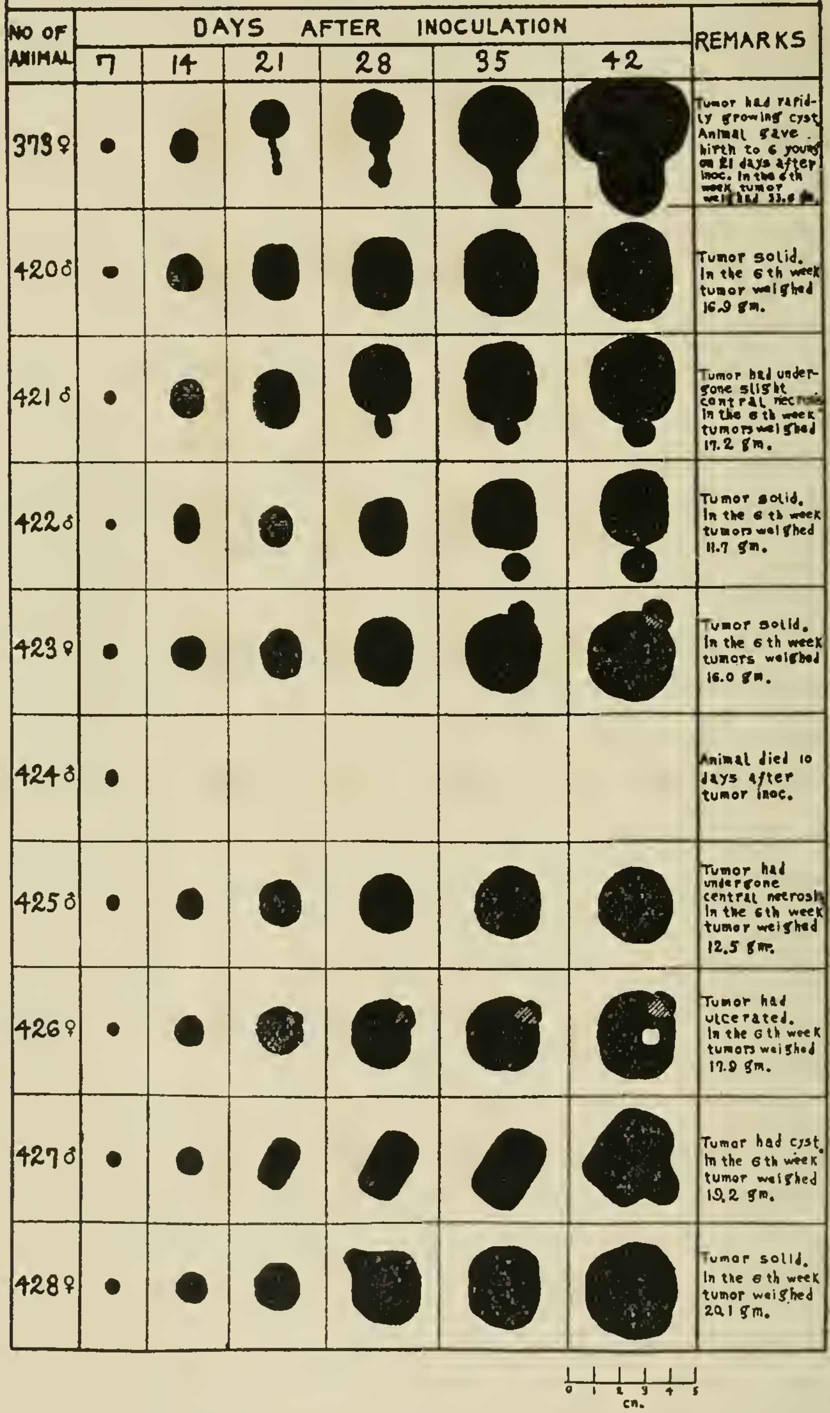
In the next experiment we made use of a diet more decidedly restricted in accessory food substances. This diet consisted of bananas, 84 per cent, and purified casein, 16 per cent. We have previously shown that such a diet is inadequate for the normal growth of young rats (26).

Experiment 4, December 18, 1918.

Seven young rats, the average weight of which was 97 grams, were fed with this special diet for two days prior to the tumor inoculation. Eight animals on normal diet were used as controls.

FIGURE V

SHOWING THE GROWTH OF THE $\frac{\text{PRC}}{660}$ IN RATS · FED UPON THE NORMAL DIET OF BREAD, MILK AND CARROTS OR CABBAGE.



NO OF ANIMAL	REMARKS
379♀	Tumor had rapidly growing cyst. Animal gave birth to 6 young on 21 days after inoc. In the 6th week tumor weighed 33.6 gm.
420♂	Tumor solid. In the 6th week tumor weighed 16.9 gm.
421♂	Tumor had undergone slight central necrosis. In the 6th week tumors weighed 17.2 gm.
422♂	Tumor solid. In the 6th week tumors weighed 11.7 gm.
423♀	Tumor solid. In the 6th week tumors weighed 16.0 gm.
424♂	Animal died 10 days after tumor inoc.
425♂	Tumor had undergone central necrosis. In the 6th week tumor weighed 12.5 gm.
426♀	Tumor had ulcerated. In the 6th week tumors weighed 17.9 gm.
427♂	Tumor had cyst. In the 6th week tumor weighed 19.2 gm.
428♀	Tumor solid. In the 6th week tumor weighed 20.1 gm.

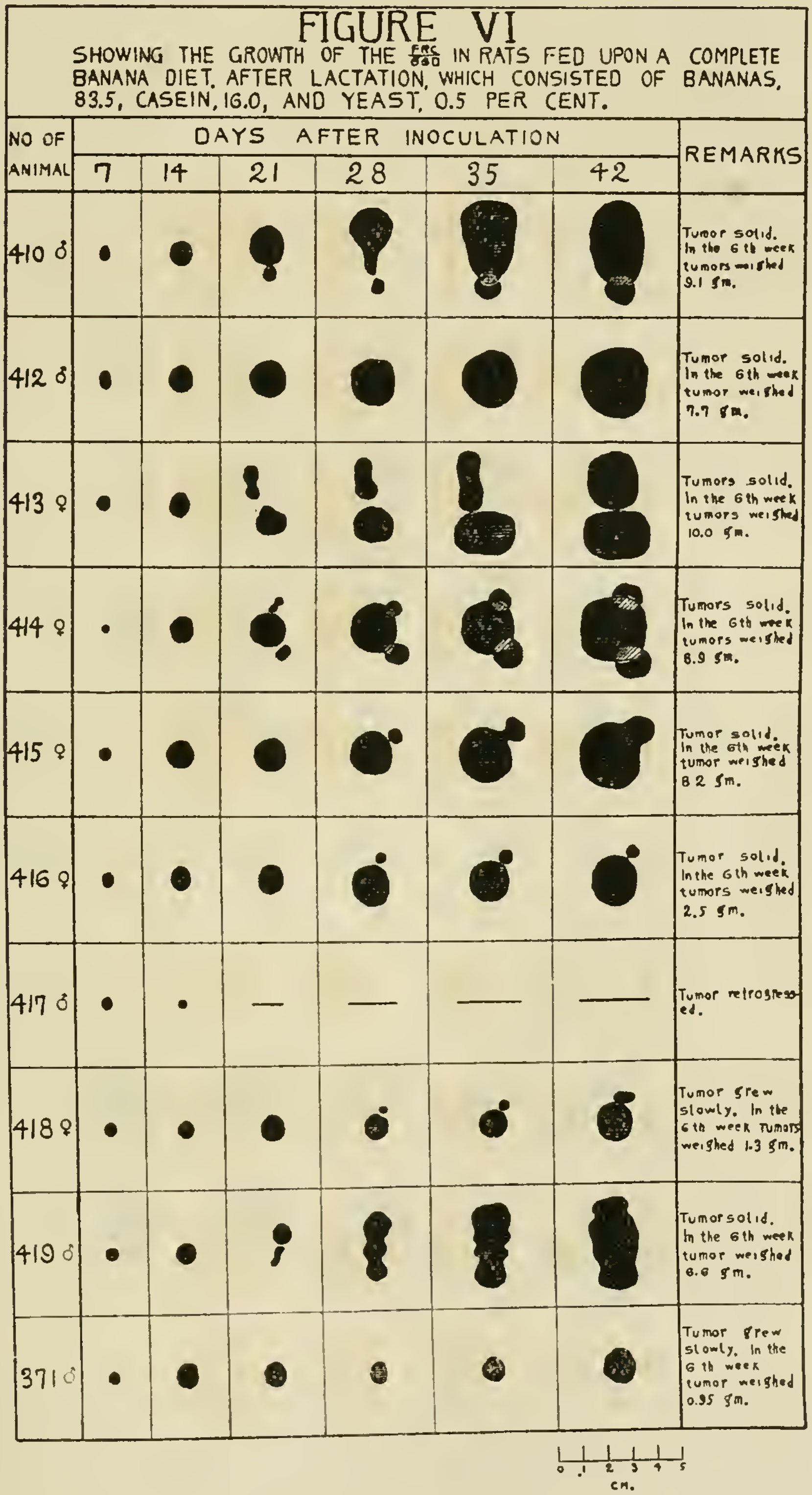


FIGURE VI

SHOWING THE GROWTH OF THE $\frac{FRC}{880}$ IN RATS FED UPON A COMPLETE BANANA DIET. AFTER LACTATION, WHICH CONSISTED OF BANANAS, 83.5, CASEIN, 16.0, AND YEAST, 0.5 PER CENT.

NO OF ANIMAL	DAYS AFTER INOCULATION 7	14	21	28	35	42	REMARKS
410 ♂							Tumor solid. In the 6 th week tumors weighed 9.1 gm.
412 ♂							Tumor solid. In the 6th week tumor weighed 7.7 gm.
413 ♀							Tumors solid. In the 6th week tumors weighed 10.0 gm.
414 ♀							Tumors solid. In the 6th week tumors weighed 8.9 gm.
415 ♀							Tumor solid. In the 6th week tumor weighed 8 2 gm.
416 ♀							Tumor solid. In the 6th week tumors weighed 2.5 gm.
417 ♂			—	—	—	—	Tumor retrogressed.
418 ♀							Tumor grew slowly. In the 6 th week Tumors weighed 1.3 gm.
419 ♂							Tumor solid. In the 6 th week tumor weighed 6.6 gm.
371 ♂							Tumor grew slowly. In the 6 th week tumor weighed 0.95 gm.

The results obtained from this experiment are shown in figures VII and VIII.

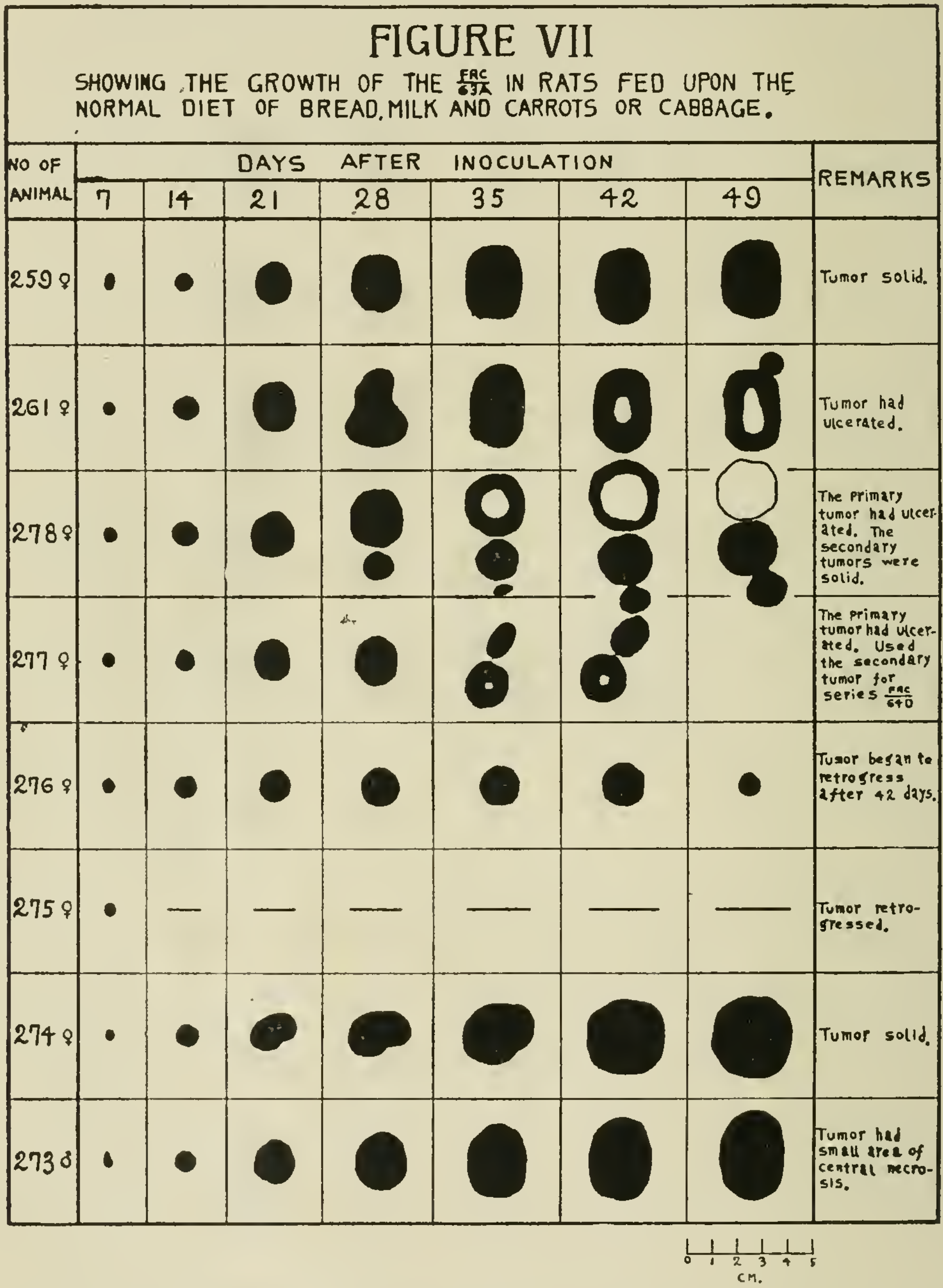


FIGURE VII

SHOWING THE GROWTH OF THE $\frac{FRC}{63A}$ IN RATS FED UPON THE NORMAL DIET OF BREAD, MILK AND CARROTS OR CABBAGE.

From these results it would be concluded that the banana-casein diet has a decidedly retarding influence upon tumor growth and an inhibitory action upon tumor susceptibility. This single experiment, however, does not warrant us in drawing a definite conclusion.

The experiment was repeated with nodules of $\frac{\text{FRC}}{\text{67E}}$.

Experiment 5, May 22, 1919.

Four young rats were placed on the banana-casein diet and eight young rats we continued feeding on our normal diet. Fifteen days later tumor bits were inoculated into these animals under aseptic precautions.

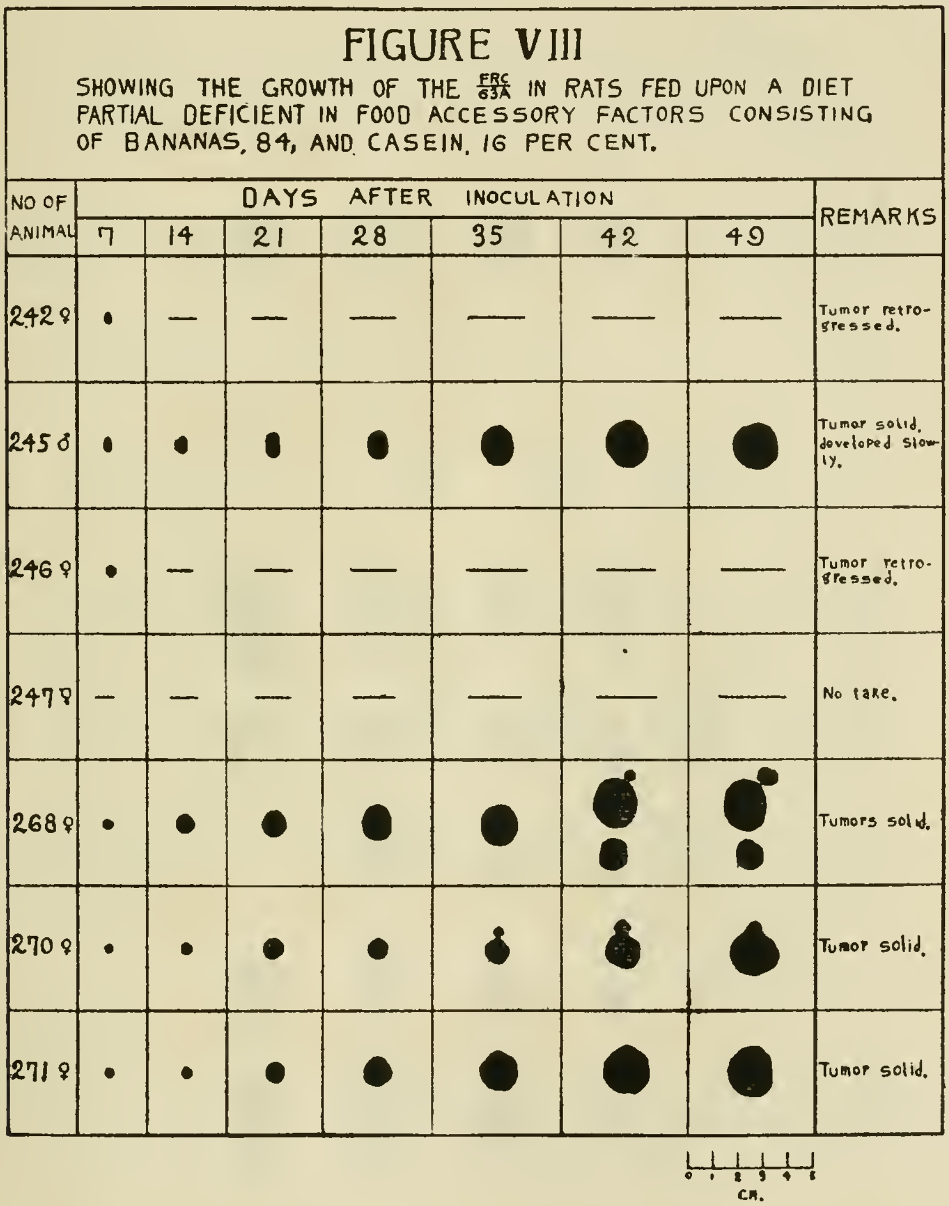


FIGURE VIII

SHOWING THE GROWTH OF THE $\frac{FRC}{63A}$ IN RATS FED UPON A DIET PARTIAL DEFICIENT IN FOOD ACCESSORY FACTORS CONSISTING OF BANANAS, 84, AND CASEIN, 16 PER CENT.

NO OF ANIMAL	DAYS AFTER INOCULATION							REMARKS
	7	14	21	28	35	42	49	
242 ♀	●	—	—	—	—	—	—	Tumor retrogressed.
245 ♂	●	●	●	●	●	●	●	Tumor solid, developed slowly.
246 ♀	●	—	—	—	—	—	—	Tumor retrogressed.
247 ♀	—	—	—	—	—	—	—	No take.
268 ♀	●	●	●	●	●	● ●	● ●	Tumors solid.
270 ♀	●	●	●	●	●	●	●	Tumor solid.
271 ♀	●	●	●	●	●	●	●	Tumor solid.

The results obtained from this experiment are recorded in figures IX and X.

It is seen that the grafted tumors grew with equal rapidity in hosts fed upon the banana-casein diet and in the controls. A difference, therefore, may exist among tumors of different generations in the nutriment required for their development.

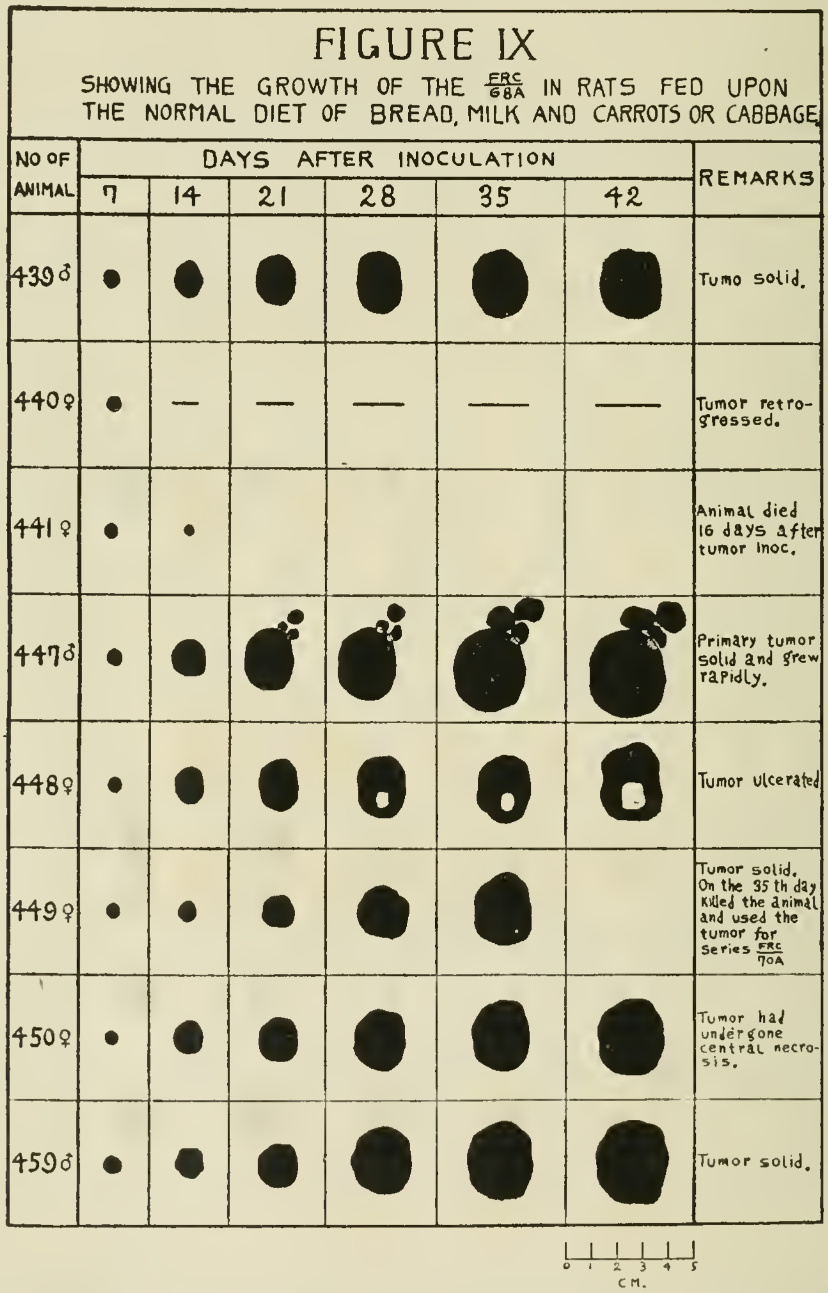


FIGURE IX

SHOWING THE GROWTH OF THE $\frac{FRC}{68A}$ IN RATS FED UPON THE NORMAL DIET OF BREAD, MILK AND CARROTS OR CABBAGE.

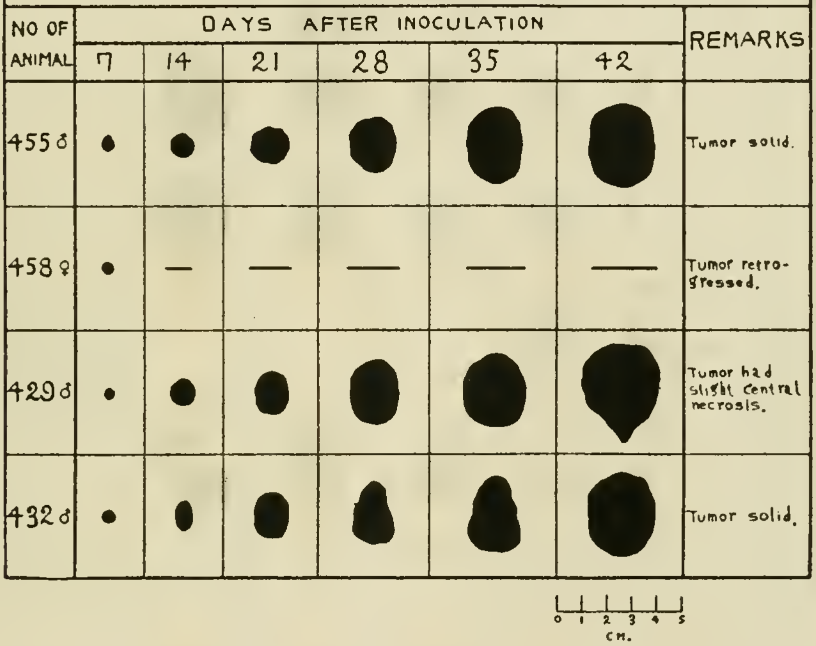
NO OF ANIMAL	DAYS AFTER INOCULATION						REMARKS
	7	14	21	28	35	42	
439♂	●	●	●	●	●	●	Tumo solid.
440♀	●	—	—	—	—	—	Tumor retrogressed.
441♀	●	●					Animal died 16 days after tumor Inoc.
447♂	●	●	●	●	●	●	Primary tumor solid and grew rapidly.
448♀	●	●	●	●	●	●	Tumor ulcerated
449♀	●	●	●	●	●		Tumor solid. On the 35 th day Killed the animal and used the tumor for Series $\frac{FRC}{70A}$
450♀	●	●	●	●	●	●	Tumor had undergone central necrosis.
459♂	●	●	●	●	●	●	Tumor solid.

In an earlier paper (26) we have shown that young albino rats have failed to maintain body weight upon an exclusive banana diet. Animals either remained at nearly constant body weight after the first day's drop, or kept losing weight steadily until death. Many animals lived upon bananas for nearly two and a half months, but did not grow. We stated that the banana is deficient in (a) protein, and (b) the water-soluble accessory factor, because the addition of definite amounts of casein and yeast to the banana resulted in rapid growth of young rats.

Three sets of experiments were made to determine the effect of bananas alone upon the growth of and susceptibility to tumor, in the following manner:

FIGURE X

SHOWING THE GROWTH OF THE $\frac{FRC}{68A}$ IN RATS FED UPON A DIET PARTIALLY DEFICIENT IN FOOD ACCESSORY FACTORS CONSISTING OF BANANAS, 84, AND CASEIN, 16 PER CENT.

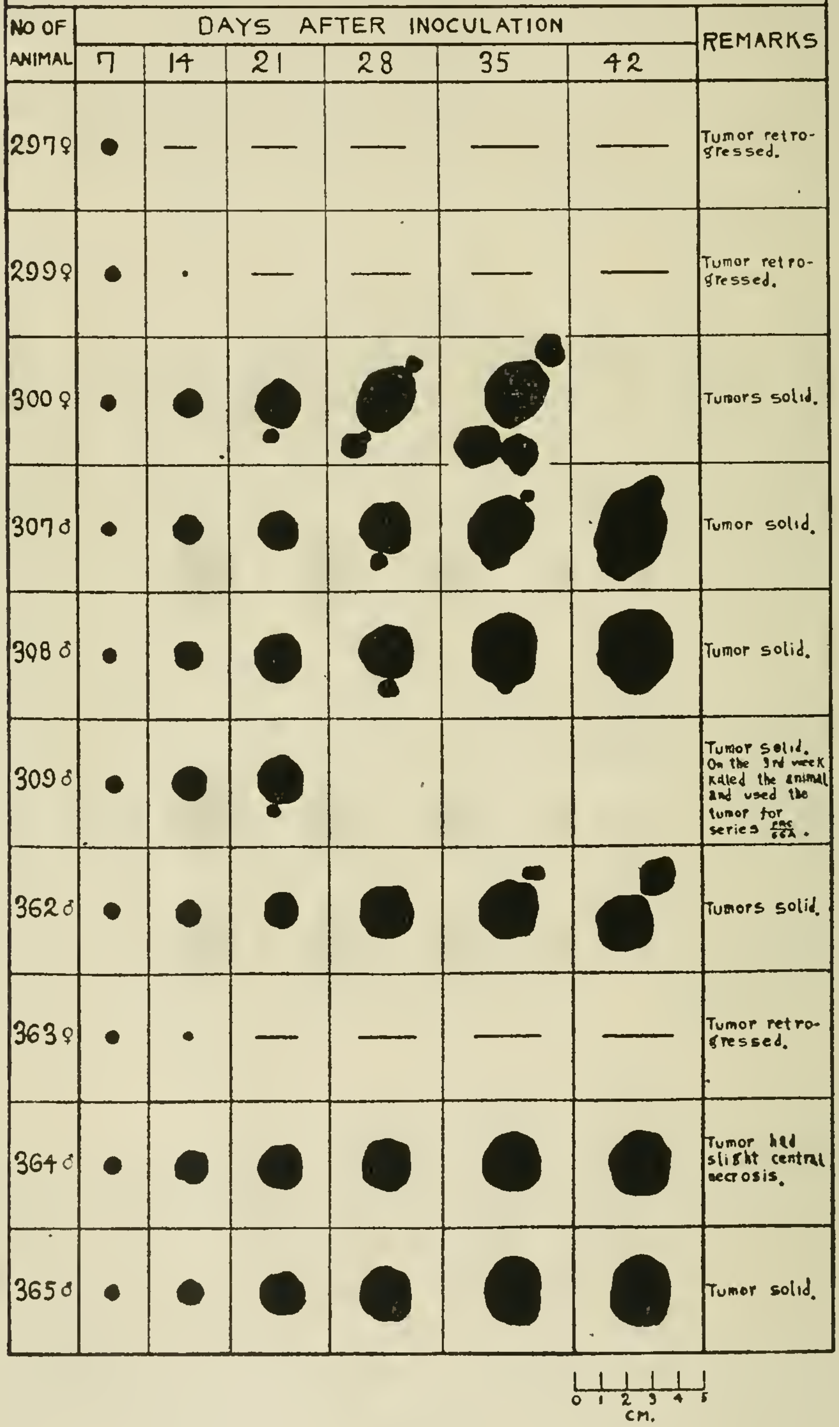


Experiment 6, February 15, 1919.

Twenty young healthy rats, the average weight of which was 78 grams, were divided into two groups. Ten of them were placed on the banana diet and the remaining ten were kept on the normal diet. Fourteen days later tumor fragments were inoculated into these animals under aseptic precautions. The results obtained from this experiment are shown in figures XI and XII.

FIGURE XI

SHOWING THE GROWTH OF THE $\frac{FRC}{65A}$ IN RATS FED UPON THE NORMAL DIET OF BREAD, MILK AND CARROTS OR CABBAGE



NO OF ANIMAL	DAYS AFTER INOCULATION						REMARKS
	7	14	21	28	35	42	
297♀	●	—	—	—	—	—	Tumor retrogressed.
299♀	●	●	—	—	—	—	Tumor retrogressed.
300♀	●	●	●	●	●		Tumors solid.
307♂	●	●	●	●	●	●	Tumor solid.
308♂	●	●	●	●	●	●	Tumor solid.
309♂	●	●	●				Tumor solid. On the 3rd week Killed the animal and used the tumor for series $\frac{FRC}{66A}$.
362♂	●	●	●	●	●	●	Tumors solid.
363♀	●	●	—	—	—	—	Tumor retrogressed.
364♂	●	●	●	●	●	●	Tumor had slight central necrosis.
365♂	●	●	●	●	●	●	Tumor solid.

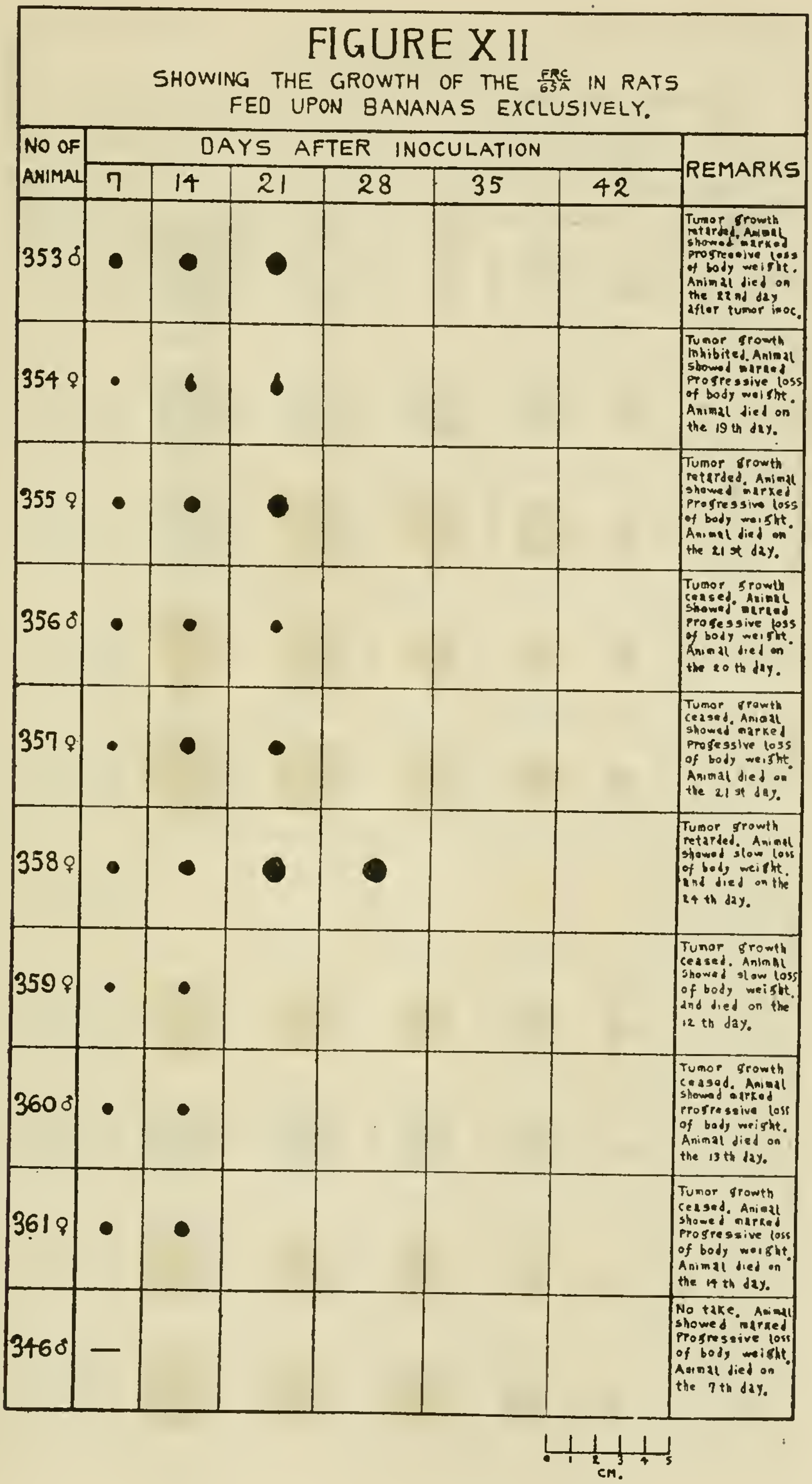


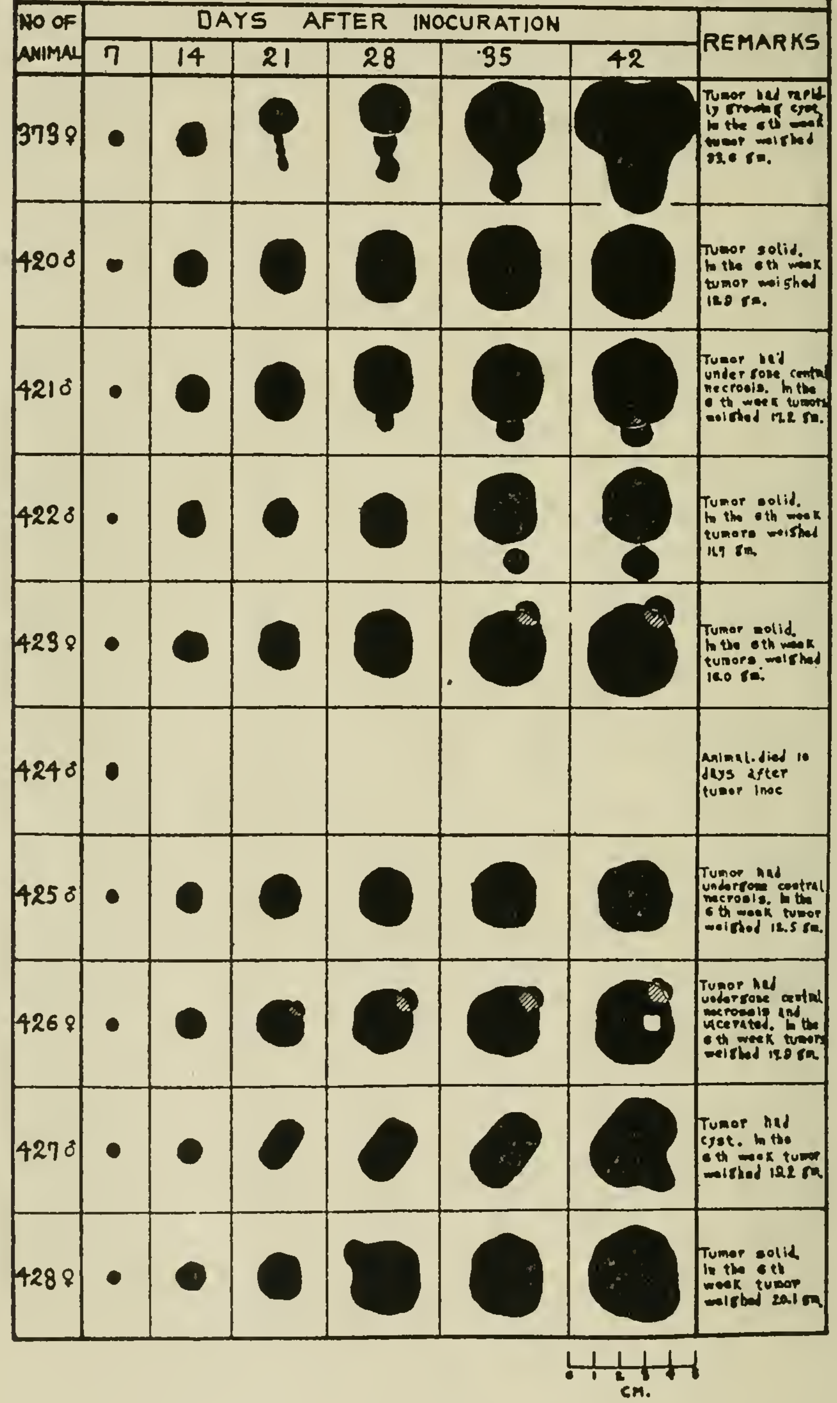
FIGURE XII

SHOWING THE GROWTH OF THE $\frac{FRC}{65A}$ IN RATS FED UPON BANANAS EXCLUSIVELY.

NO OF ANIMAL	DAYS AFTER INOCULATION 7	14	21	28	35	42	REMARKS
353♂	●	●	●				Tumor growth retarded. Animal showed marked progressive loss of body weight. Animal died on the 22nd day after tumor inoc.
354♀	●	●	●				Tumor growth inhibited. Animal showed marked progressive loss of body weight. Animal died on the 19th day.
355♀	●	●	●				Tumor growth retarded. Animal showed marked progressive loss of body weight. Animal died on the 21st day.
356♂	●	●	●				Tumor growth ceased. Animal showed marked progessive loss of body weight. Animal died on the 20th day.
357♀	●	●	●				Tumor growth ceased. Animal showed marked progessive loss of body weight. Animal died on the 21st day.
358♀	●	●	●	●			Tumor growth retarded. Animal showed slow loss of body weight. and died on the 24th day.
359♀	●	●					Tumor growth ceased. Animal showed slow loss of body weight. and died on the 12th day.
360♂	●	●					Tumor growth ceased. Animal showed marked progressive loss of body weight. Animal died on the 13th day.
361♀	●	●					Tumor growth ceased. Animal showed marked progressive loss of body weight. Animal died on the 14th day.
346♂	—						No take. Animal showed marked progressive loss of body weight. Animal died on the 7th day.

FIGURE XIII

SHOWING THE GROWTH OF THE $\frac{FRC}{66D}$ IN RATS FED UPON THE NORMAL DIET OF BREAD, MILK AND CARROTS OR CABBAGE.



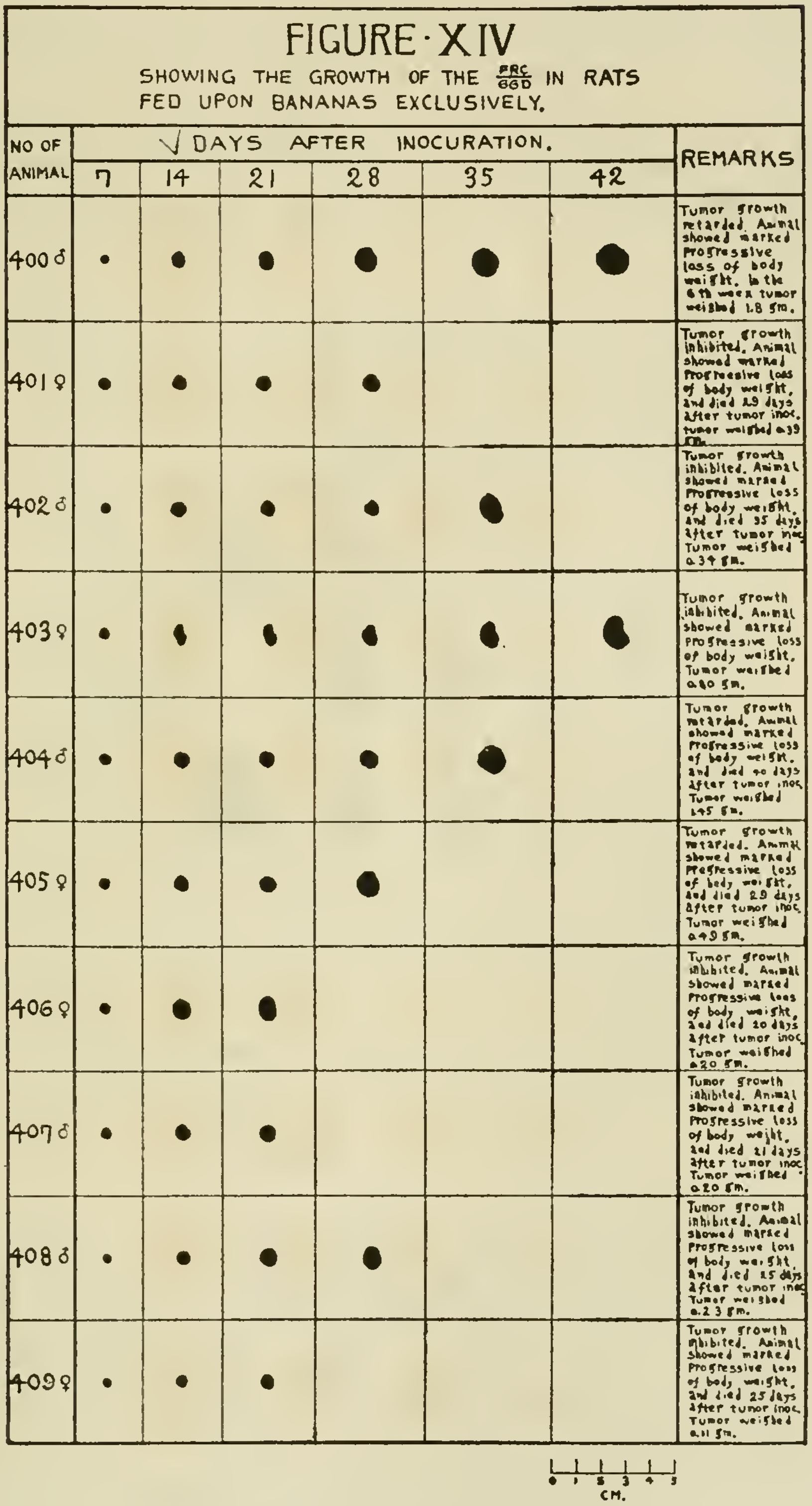


FIGURE · XIV
SHOWING THE GROWTH OF THE $\frac{FRC}{66D}$ IN RATS
FED UPON BANANAS EXCLUSIVELY.

NO OF ANIMAL	√ DAYS AFTER INOCURATION. 7	14	21	28	35	42	REMARKS
400 ♂	●	●	●	●	●	●	Tumor growth retarded. Animal showed marked progressive loss of body weight. In the 6th week tumor weighed 1.8 gm.
401 ♀	●	●	●	●			Tumor growth inhibited. Animal showed marked progressive loss of body weight, and died 29 days after tumor inoc. tumor weighed 0.39 gm.
402 ♂	●	●	●	●	●		Tumor growth inhibited. Animal showed marked progressive loss of body weight, and died 35 days after tumor inoc. Tumor weighed 0.34 gm.
403 ♀	●	●	●	●	●	●	Tumor growth inhibited. Animal showed marked progressive loss of body weight. Tumor weighed 0.80 gm.
404 ♂	●	●	●	●	●		Tumor growth retarded. Animal showed marked progressive loss of body weight. and died 40 days after tumor inoc. Tumor weighed 1.45 gm.
405 ♀	●	●	●	●			Tumor growth retarded. Animal showed marked progressive loss of body weight, and died 29 days after tumor inoc. Tumor weighed 0.49 gm.
406 ♀	●	●	●				Tumor growth inhibited. Animal showed marked progressive loss of body weight, and died 20 days after tumor inoc. Tumor weighed 0.20 gm.
407 ♂	●	●	●				Tumor growth inhibited. Animal showed marked progressive loss of body weight, and died 21 days after tumor inoc. Tumor weighed 0.20 gm.
408 ♂	●	●	●	●			Tumor growth inhibited. Animal showed marked progressive loss of body weight, and died 25 days after tumor inoc. Tumor weighed 0.23 gm.
409 ♀	●	●	●				Tumor growth inhibited. Animal showed marked progressive loss of body weight, and died 25 days after tumor inoc. Tumor weighed 0.11 gm.

Experiment 7, April 8, 1919.

Ten rats, ranging from 59 to 133 grams, were placed on the banana diet fourteen days prior to tumor inoculation, and the diet continued. Ten controls, ranging from 50 to 139 grams, were fed on the normal diet before and after the tumor inoculation. The results obtained from this experiment are shown in figures XIII and XIV.

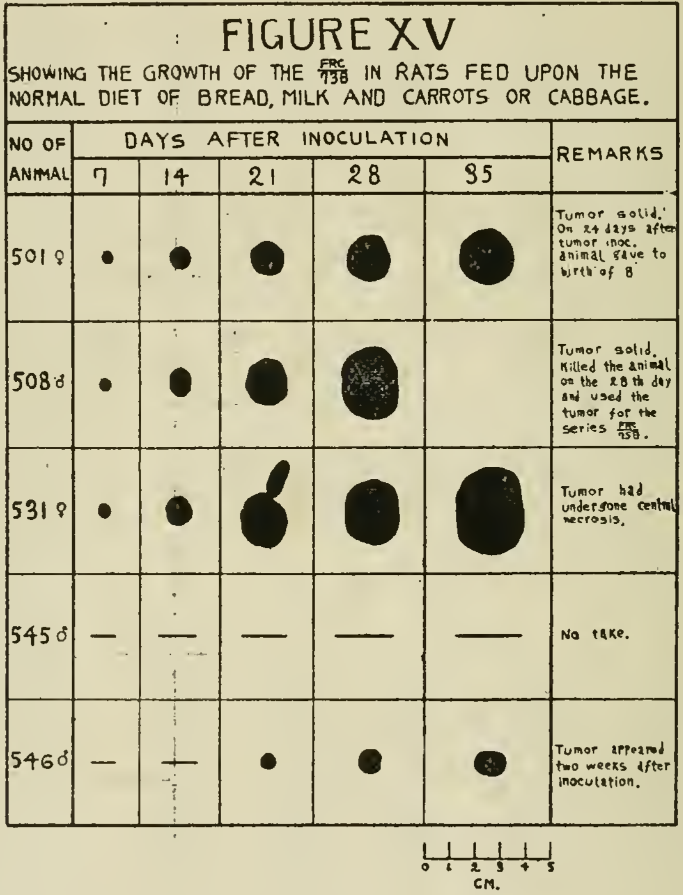


FIGURE XV

SHOWING THE GROWTH OF THE $\frac{FRC}{138}$ IN RATS FED UPON THE NORMAL DIET OF BREAD, MILK AND CARROTS OR CABBAGE.

Experiment 8, September 15, 1919.

Four of nine vigorous rats were changed to the banana diet from the normal diet seven days before the tumor inoculation, while the other five animals were allowed to remain on the normal diet. The results obtained from this experiment are shown in figures XV and XVI.

From these experiments it is plain that the banana diet had a marked retarding action upon the growth of the tumor grafts. The presence of tumor fragments in banana-fed animals hastened their death. The retarding influence of the banana diet upon transplanted tumors is less marked if the normal diet of the animals is changed to a banana diet within seven days previous to the inoculation. The banana diet has no specific influence upon tumor susceptibility.

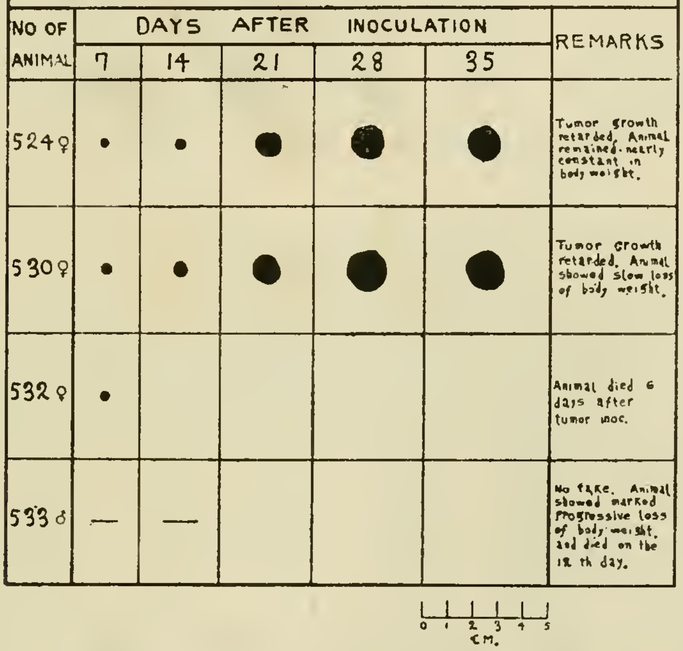
Further proof of the inhibitory action of bananas upon the growth of tumor grafts is shown in the following two experiments.

Experiment 9, May 19, 1919.

In the first experiment the normal diet of tumor-bearing animals was changed to an exclusive banana diet at the fourteenth day after tumor inoculation, the time when grafted tumors begin to develop more rapidly (see Chart I). The results from this experiment are given in figures XVII and XVIII.

FIGURE XVI

SHOWING THE GROWTH OF THE [illegible] IN RATS FED UPON BANANAS EXCLUSIVELY.



NO OF ANIMAL	DAYS AFTER INOCULATION 7	14	21	28	35	REMARKS
524♀	•	•	●	●	●	Tumor growth retarded. Animal remained nearly constant in body weight.
530♀	•	•	●	●	●	Tumor growth retarded. Animal showed slow loss of body weight.
532♀	•					Animal died 6 days after tumor inoc.
533♂	—	—				No take. Animal showed marked progressive loss of body weight, and died on the 12 th day.

Experiment 10, September 18, 1919.

The second experiment was just the reverse of the preceding. Five of nine tumor-bearing animals fed upon exclusive banana diet were given normal diet fourteen days after tumor inoculation. The results are shown in figures XIX and XX.

FIGURE XVII

SHOWING THE GROWTH OF THE $\frac{FRC}{67E}$ IN RATS FED UPON THE NORMAL DIET OF BREAD, MILK AND CARROTS OR CABBAGE.

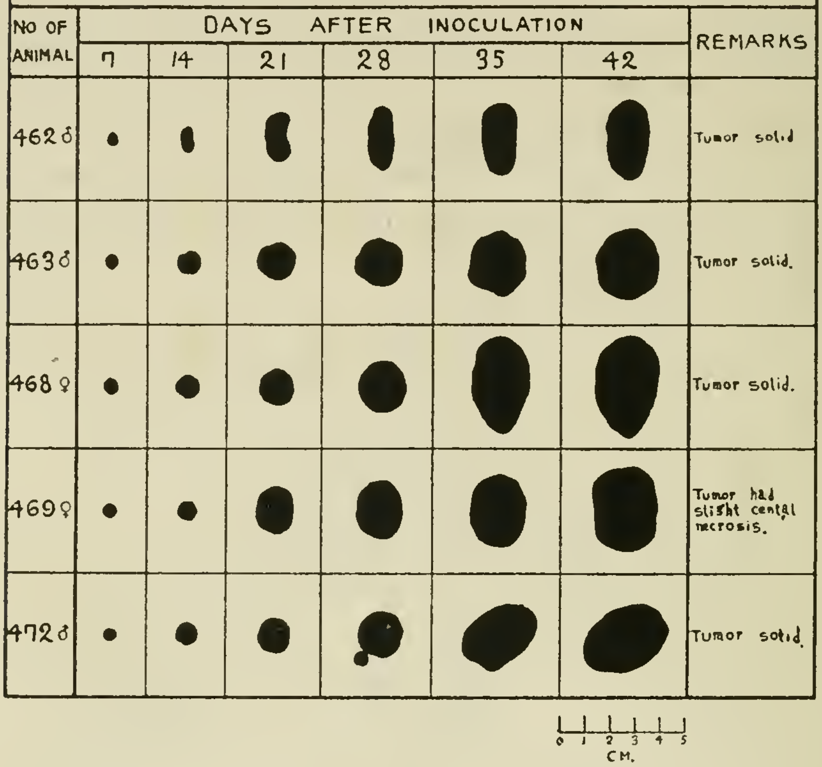
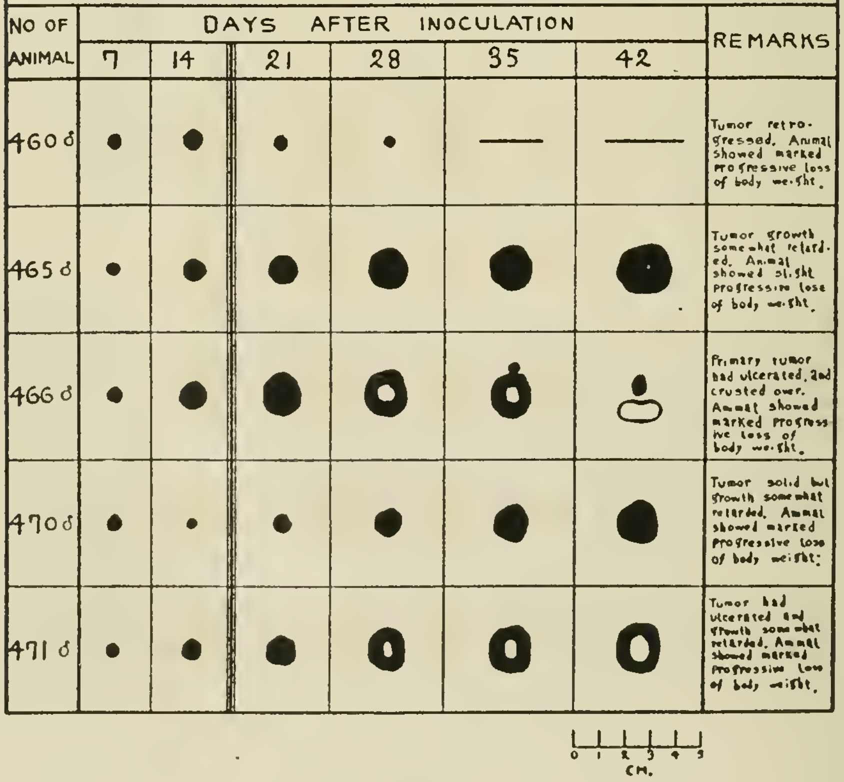


FIGURE XVIII

SHOWING THE GROWTH OF THE $\frac{FRC}{67E}$ IN RATS FED UPON THE NORMAL DIET OF BREAD, MILK AND CARROTS OR CABBAGE UNTIL TWO WEEKS AFTER TUMOR INOCULATION, THEN ON AN EXCLUSIVE BANANA DIET.



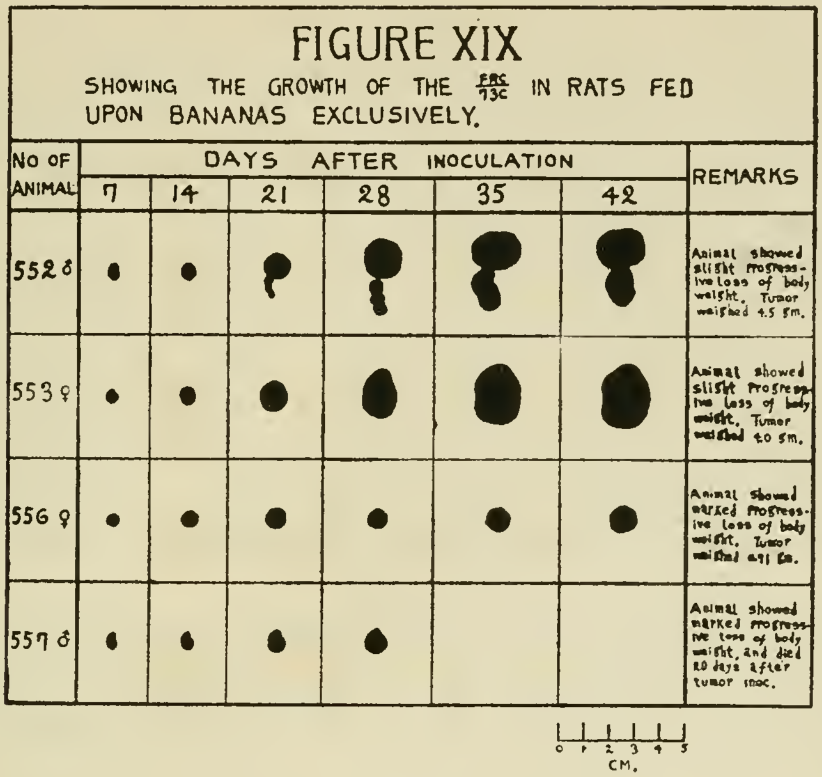


FIGURE XIX

SHOWING THE GROWTH OF THE $\frac{FRC}{73C}$ IN RATS FED UPON BANANAS EXCLUSIVELY.

No OF ANIMAL	DAYS AFTER INOCULATION 7	14	21	28	35	42	REMARKS
552♂							Animal showed slight progressive loss of body weight. Tumor weighed 4.5 gm.
553♀							Animal showed slight progressive loss of body weight. Tumor weighed 10 gm.
556♀							Animal showed marked progressive loss of body weight. Tumor weighed .91 gm.
557♂							Animal showed marked progressive loss of body weight, and died 20 days after tumor inoc.

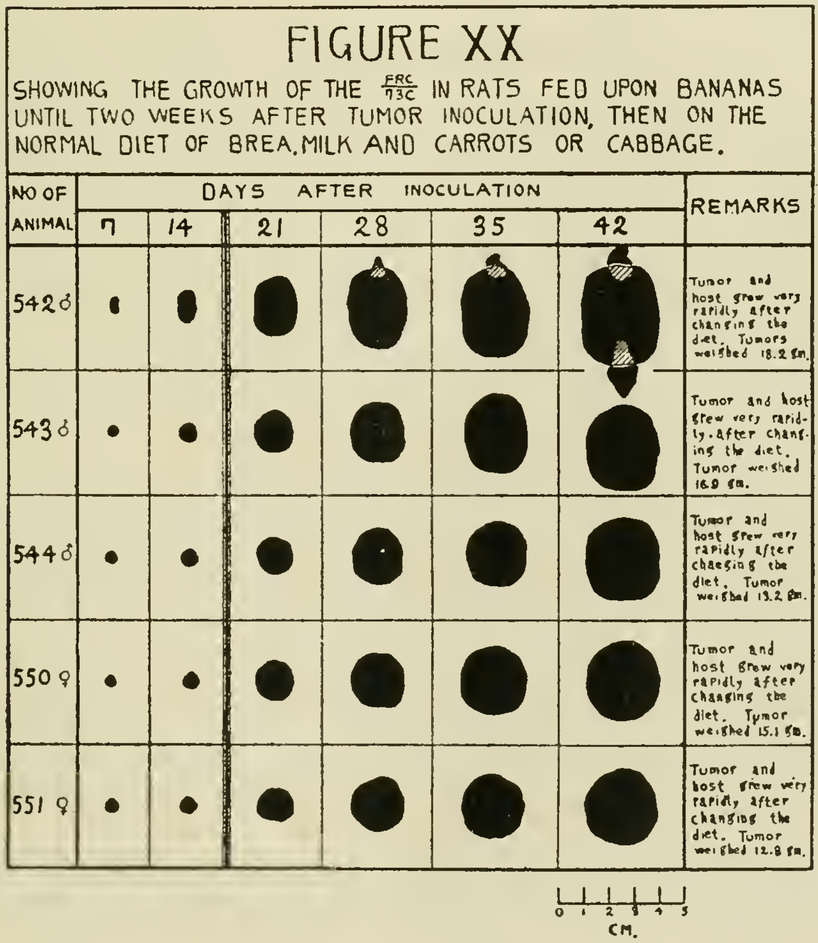


FIGURE XX

SHOWING THE GROWTH OF THE $\frac{FRC}{73C}$ IN RATS FED UPON BANANAS UNTIL TWO WEEKS AFTER TUMOR INOCULATION, THEN ON THE NORMAL DIET OF BREA, MILK AND CARROTS OR CABBAGE.

No OF ANIMAL	DAYS AFTER INOCULATION 7	14	21	28	35	42	REMARKS
542♂							Tumor and host grew very rapidly after changing the diet. Tumors weighed 18.2 gm.
543♂							Tumor and host grew very rapidly. After changing the diet. Tumor weighed 16.9 gm.
544♂							Tumor and host grew very rapidly after changing the diet. Tumor weighed 13.2 gm.
550♀							Tumor and host grew very rapidly after changing the diet. Tumor weighed 15.1 gm.
551♀							Tumor and host grew very rapidly after changing the diet. Tumor weighed 12.8 gm.

Rats 460, 465, 466, 470, and 471 of the first part of the present experiments lost steadily in body weight when their normal diet was changed to an exclusive banana diet. Their tumors grew, but not at the normal rate. It appears that the tissues of the hosts supplied nutrient material to the tumors.

Rats 542, 543, 544, 550, and 551 of the second part of the present investigations grew supernormally for the first few days after they were returned to a complete diet. Also the development of the tumor nodules was very rapid and reached to the normal sizes for the third, fourth, fifth, and sixth week respectively.

We have successfully inoculated tissues of a stunted tumor taken from a rat fed upon exclusive banana diet into young rats that were feeding upon normal diet.

These experiments give evidence that our diets did not alter the malignant character of the Flexner-Jobling rat carcinoma, though in some instances the diets markedly diminished the rate of growth.

HISTOLOGICAL OBSERVATIONS

Dr. James Ewing has kindly examined microscopically our tumor specimens[2] obtained from rats fed upon normal diet of bread, milk, and carrots or cabbage, and those fed upon our special banana diets, namely, banana-casein-yeast-protein-free milk; banana-casein-yeast; banana-casein; and banana alone.

He has drawn the following general conclusions:

1. General structure of tumors from rats fed upon normal diet and a complete banana diet is identical.
2. The stunted tumors taken from rats fed upon exclusive banana diet show the presence of well nourished cells, free from areas of necrosis and generally free from fibrosis, indicating full vitality. In other words, the tumors of banana-fed animals are as well nourished as those of the controls.

It is interesting to note that these histological findings corroborate the experimental results in showing that the malignant character of the growth was not changed by the banana diet.

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[2] The writers wish to express their appreciation to Mrs. A. Punshon of Memorial Hospital, who has kindly prepared the histological material.

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EFFECT OF A REDUCTION OF LYMPHOCYTES ON THE GROWTH RATE OF TRANSPLANTED SPONTANEOUS TUMORS IN MICE

FREDERICK PRIME

From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director

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Recently much interest has been centered upon the hypothesis that the variation in the number of the hemal lymphocytes may exert an important immunal function, diminishing or increasing the resistance of animals to tumor inoculations. Experimentally an increase or decrease in these cells is most easily induced by *x*-rays, and with the improved modern technique more accurate quantitative estimations of the rays are obtainable than was possible a few years ago. We owe to Murphy and Ellis the observation that small doses of *x*-ray repeated over consecutive days reduce the lymphocyte count, without seriously affecting the health of the animal (1). The experiments hitherto conducted on this phase of the cancer problem have been largely directed to the study of the effects of lymphocytic alterations upon transplantable tumors, where growth had already been well established. Sittenfield (2), using the Flexner-Jobling rat carcinoma, a transplantable tumor which has been observed through many generations, found that neither increase nor reduction of the lymphoid elements in the blood had any influence upon either resistance or susceptibility to tumor growth, an observation which he confirmed in a later series of experiments (3). What influence, if any, might be observed upon the growth of spontaneous tumors when transplanted into such *x*-rayed animals has not as yet been satisfactorily determined. Murphy and Morton (4) found that such tumors transplanted to *x*-rayed animals "acted much as heteroplastic tissues, retrogressing as the lymphoid tissue regenerates," and they also assert that the resistance of animals to the implantation of their own spontaneous tumor is increased by stimulating the production of lymphocytes with x-ray (5). Chambers, Scott, and Russ (6) have also reported experiments on one primary mouse tumor only. They observed a lowering of an animal's resistance to implantation after a single large generalized dose of x-ray. Since, however, a large proportion of spontaneous tumors either do not grow when transplanted into other animals or give a very small percentage of takes, it seemed of practical importance to test the other aspect of the problem and to ascertain whether resistance to implantation can be decreased by a reduction of the lymphocytes. If this were possible, it would provide an easy method of propagating many tumors of value in cancer research, which otherwise would be lost owing to our inability to transplant them successfully.

The spontaneous tumors used were derived chiefly from a special strain of "Lathrop" animals, of which this laboratory had a large number. They were inoculated into mice from the same "Lathrop" strain, as such a closely related strain offers a more favorable soil than stock of another breed. Forty-eight to seventy-two animals in each case were used, twenty-four being set aside as controls, and twenty-four to forty-eight kept for x-ray treatment.

To prepare the animals for inoculation they were given seven consecutive x-ray treatments according to Murphy's directions for reducing the number of lymphocytes. At the end of this time a spontaneous tumor was removed from its host and a small piece, about 0.002 gram in weight, was inoculated into the right groin of both the untreated controls and the mice whose lymphocytes had been greatly reduced by x-ray. Before starting the x-ray treatment, as well as twenty-four hours after the last dose of x-ray had been given, and at weekly intervals thereafter for six weeks, the blood of the x-rayed mice as well as that of the controls was counted. The blood pictures all showed a marked fall in the lymphocyte count after the administration of seven consecutive doses of x-ray, with a gradual return to around the normal point by the end of six weeks. The controls during the early part of the experiment all showed a marked spontaneous increase in the lymphocyte count.

The experiments lasted ten weeks or more, no animals being included in the last series of counts which had not survived for this period, and many of the series were observed over a period of twenty weeks. The results were quite different from those reported by Murphy, who used tumors which had been transplanted for many generations. He records that a reduction of the lymphocytes in the circulating blood was attended by an increase in the number of takes. In our experiments there was found to be practically no difference at all in the inoculation percentage between the untreated controls and those which had been given a reducing dosage of x-ray. The lymphocyte count in many of the controls was higher at the end than at the beginning of the experiment, and there should, therefore, according to the DaFano-Murphy theory, have been a smaller number of takes among them; but this was not the case.

Out of a series of 905 animals living at the end of five weeks which had been treated with x-ray, 72, or 7.9 per cent, showed tumors, and 92.1 per cent were negative; while out of 740 controls which were living, 6 per cent showed tumors, and 94 per cent were negative. At the end of ten weeks, out of 740 living animals which had been treated with x-ray, 4.3 per cent showed tumors, and 95.7 per cent showed no growths at all; and out of 615 controls, 3.7 per cent of the animals showed tumor growths, and 96.3 per cent were negative. A difference of 2 per cent in the number of takes at the end of five weeks between the animals whose lymphocytes had been reduced and the controls has no significance, and a difference of 0.6 per cent at the end of ten weeks shows how little evidence there is in favor of the theory that the lymphocyte plays any important part in the production of immunity in relation to transplanted cancer (see chart 1). If the DaFano-Murphy hypothesis that the lymphocyte plays an important rôle in the production of immunity were correct, a reduction in the number of tumor takes should have occurred in these experiments; but, as has been shown, such was not the case.

That the x-ray treatment was sufficient to reduce materially the number of lymphocytes in the circulating blood was clearly demonstrated by the blood counts; and at the end of four weeks, ample time for some evidence of tumor growth to be seen, the blood had not yet returned to normal. Whether this condition applies to spontaneous tumors alone, or to transplantable tumors in general, will have to be demonstrated for each tumor.

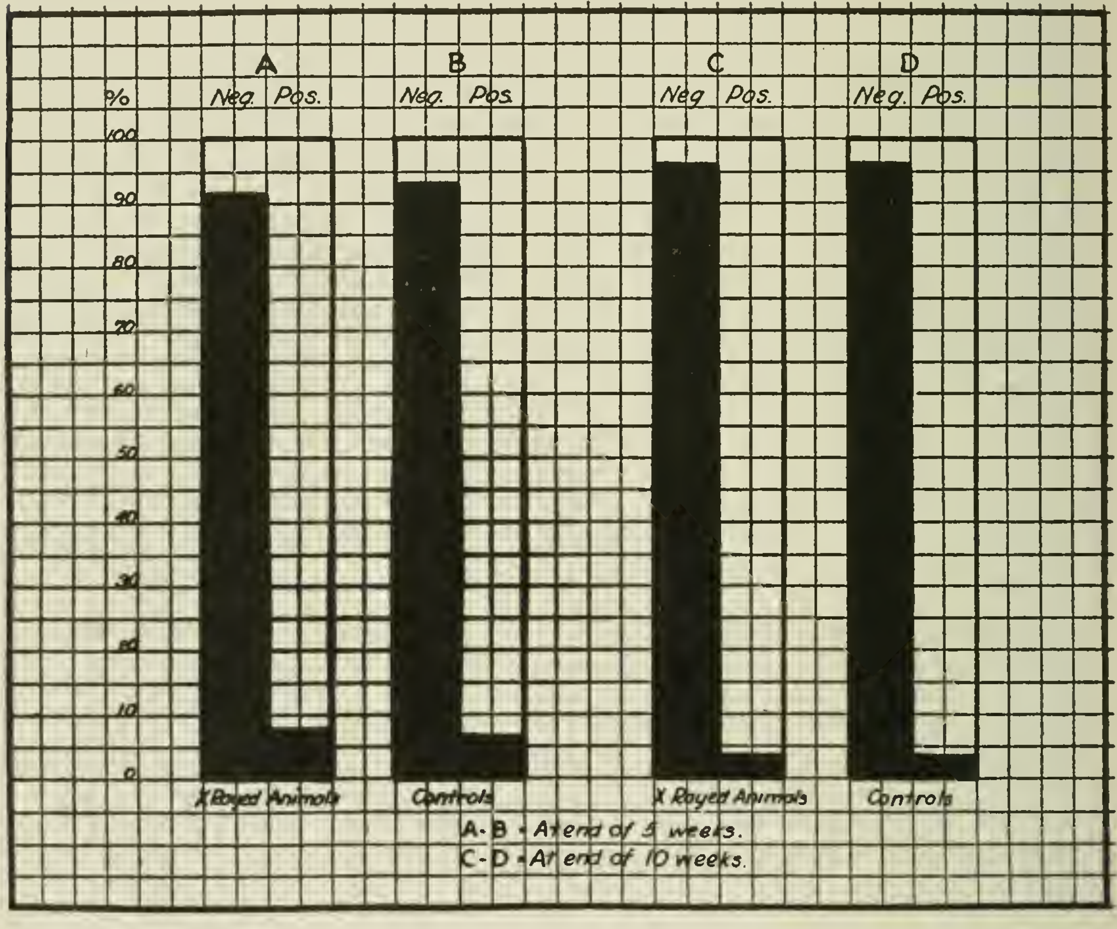

Chart 1

In the series reported, over forty spontaneous tumors were used and inoculations were made into 2100 mice.

CONCLUSIONS

Decreasing the circulating lymphocytes by small doses of x-ray does not render mice more susceptible to the inoculation of a spontaneous tumor from another mouse of the same strain.

Mice with spontaneous lymphocytosis are not resistant to the implantation of a primary tumor from another mouse of the same strain.

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A COMPARISON OF THE GROWTH OF MICE WHICH ULTIMATELY DEVELOP CARCINOMA WITH THE GROWTH OF MICE WHICH DO NOT DEVELOP CARCINOMA

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STATEMENT OF THE PROBLEM

Since cancer is a disease of growth, and its incidence in appreciable form is usually deferred until after the attainment of maturity, a very important factor in our ultimate understanding of the etiology of cancer must arise out of a knowledge of the degree to which the preceding development of the animal is affected by the ultimate aberration of growth. If the factors which determine the growth and incidence of carcinoma, for example, are of an essentially accidental character, such as local irritation *plus* infection, then the incidence of carcinoma in an individual will be decided in the main by factors not inherent in its habit of growth and metabolism, and the preceding normal development cannot be expected to foreshadow the impending pathological outcome. If, on the contrary, the incidence of carcinoma is determined by internal factors of growth-habit or metabolic peculiarities *plus* recurrent external factors, such as local irritation, then the underlying peculiarity of growth or metabolism may conceivably reveal itself in more or less marked departures from the average in the preceding normal development.

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[1] The expenses of this research were in part defrayed by a grant from the special medical research fund of the University of Toronto.

A comparison throughout the duration of life of the growth of animals which ultimately develop spontaneous cancer and those which do not is, therefore, fundamental to our comprehension of the etiology of this disease. The fact that such a comparison has not hitherto been instituted is undoubtedly attributable to the experimental labor which is essential to obtain results of any worth, to the expenditure of time which is requisite, and to the difficulty of an experimental technique which must obviously be devised to exclude all chance causes of death, among which must be reckoned epidemic infections. It is obvious that if epidemic infections were from time to time to claim a substantial proportion of victims among the experimental animals, it would become impossible to identify the individuals which, had they survived the infection, would subsequently have developed carcinoma.

METHODS OF RESEARCH

For the past six years we have been engaged upon an extensive series of experiments of which the immediate object was the determination of the influence of certain glandular extracts or of normal food constituents in extraordinary excess upon the growth of otherwise normal white mice. The unusual constituent was added to an otherwise varied and abundant diet of barley, egg, biscuit, and lettuce. It was essential to our inquiry that epidemic infections should be excluded, and we were so far successful that mortality from these diseases, throughout the duration of their lives, did not amount to more than 4 per cent of the total deaths in the males, and 1 per cent in the females. The animals were weighed once a week from the fourth or fifth week after birth until the thirtieth week, and thereafter once a fortnight. Individual records of the growth of each mouse were kept, and its ultimate fate recorded. All lesions manifest to the naked eye in postmortem examination of the organ were noted, and sections of formaldehyde-hardened material were prepared and examined microscopically. We feel confident that nearly all cases of carcinoma and other new growths were detected and identified except in a very small proportion of cases in which postmortem examination of the tissues was rendered impossible by delayed observation of death and consequent decomposition.

The details of our experimental technique and the growth and mortality statistics thus obtained have been fully described elsewhere (1).

By segregating, in each experimental group, the records of those animals which ultimately developed carcinoma, we have been able to compare the growth of those animals which developed carcinoma with the growth of those animals which did not, in eleven different experimental groups, involving a total of 324 animals, of which 105 developed spontaneous carcinoma. The primary growth was usually situated in the axilla or the groin, and metastases occurred in the lungs. In a small proportion of cases in the other groups and in every case in the group receiving tethelin throughout the duration of life (tethelin-fed males) the only new growths detectable by naked eye examination were situated in the lung; and these, in the tethelin-fed group, were very small, frequently but 1 to 4 mm. in diameter. In the more typical cases, involving a primary growth in the axilla or groin, death usually occurred within one month of the appearance of a manifest lump.

The diet of the "normal" animals consisted of crushed barley and water *ad libitum*, fresh leaves of lettuce twice a week, dry hard unsweetened biscuit ("pilot bread") once a week, and 5 cc. of mixed white and yolk of egg to every six animals on each of six days of the week. The egg was employed as the vehicle for the administration of the various dietary additions to the other groups; it was generally consumed within a few minutes of its introduction into the cage.

The pituitary-fed animals received one-half of an anterior lobe of an ox pituitary per six animals, emulsified in the daily allowance of egg. The lecithin, prepared from yolks of eggs, was administered in dosage corresponding to 83 mgm. per mouse per day. The dosage of cholesterol was 42 mgm. per mouse per day. The dosage of tethelin (a lipin prepared from the anterior lobe of the pituitary gland) was 4 mgm. per mouse per day. In one group (tethelin-fed males) the administration of tethelin was continued throughout life. To one group of females ("discontinuous administration") this dose of tethelin was administered in three periods of one month each, namely from the end of the fourth week until the end of the eighth, from the end of the twenty-first until the end of the twenty-fifth, and from the end of the forty-second until the end of the forty-sixth week of life. To another group of females ("brief administration") the administration was continued only from the end of the fourth until the end of the twelfth week, and thus ceased before or at about the attainment of sexual maturity. Weighings, in this latter group, were discontinued between the thirtieth and the seventieth week because, mistakenly as events proved, no noteworthy effects of the administration upon normal growth subsequent to the thirtieth week were anticipated.

EXPERIMENTAL RESULTS

The incidence of carcinoma in the various experimental classes and the average duration of life of the animals in each class and of those which developed carcinoma are enumerated in table 1.[2] It will be observed that the percentage of incidence of carcinoma was substantially unaffected by the various dietaries employed, with the exception that the incidence was extraordinarily low (17 per cent) in the group of females which received the "brief administration" of tethelin. Traces of the same effect may be seen in the low incidence (25 per cent) in the group which received "discontinuous administration" of tethelin, the normal incidence in females being, for the strain employed, from 30 to 50 per cent. We shall see that of all the growth curves herein presented that for the "brief administration" tethelin group departed most widely from the type of growth curve generally displayed by animals which subsequently developed carcinoma. The high incidence of carcinoma in the pituitary-fed groups may possibly be fortuitous, or, again, may be attributable to the pituitary tissue, or, yet again, to the fact that the diet of these animals differed from that of all other classes in containing a proportion of raw meat.

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[2] Owing to the cost of publication it has been necessary to omit many tables bearing in detail the data of the experiments.—*Editor.*

The results of the comparison of the growth of the animals which ultimately developed carcinoma with the growth of the animals which did not are displayed graphically in Figures 1 to 11, in which the full lines represent the growth of the animals which did not, and the broken lines represent the growth of the animals which did develop carcinoma. The comparison is discontinued in each case at the date at which half of the carcinoma animals had died.

TABLE 1

Incidence of carcinoma in the various experimental classes and the duration of life of each

CLASS OF ANIMALS	ANIMALS WHICH DEVELOPED CARCINOMA	AVERAGE DURATION OF LIFE OF ALL ANIMALS	AVERAGE DURATION OF LIFE OF THE ANIMALS WHICH DEVELOPED CARCINOMA
Males			
	per cent	*days*	*days*
Normal	25	767	826
Pituitary	29	792	823
Lecithin	22	731	743
Cholesterol	26	764	848
Tethelin	35	866	958
Females			
Normal	37	719	721
Pituitary	51	704	689
Lecithin	32	731	743
Cholesterol	50	658	590
Tethelin (discontinuous administration)	25	800	970
Tethelin (brief administration)	17	695	677

An examination of these curves reveals a striking uniformity of result. In each of the eleven different experimental groups the carcinomatous animals were superior in weight to the noncarcinomatous throughout a large proportion of their lives, and this occurred independently of whether, at the beginning of the weighings at four or five weeks of age, they were superior, equal,

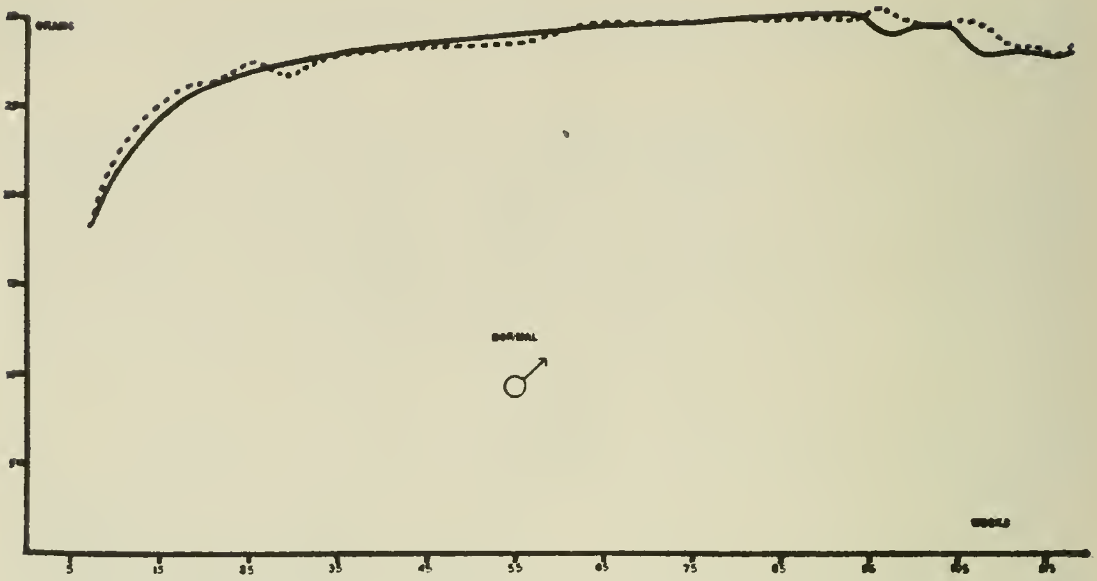

FIG 1. COMPARISON OF THE GROWTH OF NORMAL MALE WHITE MICE WHICH ULTIMATELY DEVELOPED CARCINOMA (BROKEN LINE) WITH THAT OF NORMAL MALE WHITE MICE WHICH DID NOT DEVELOP CARCINOMA (FULL LINE)

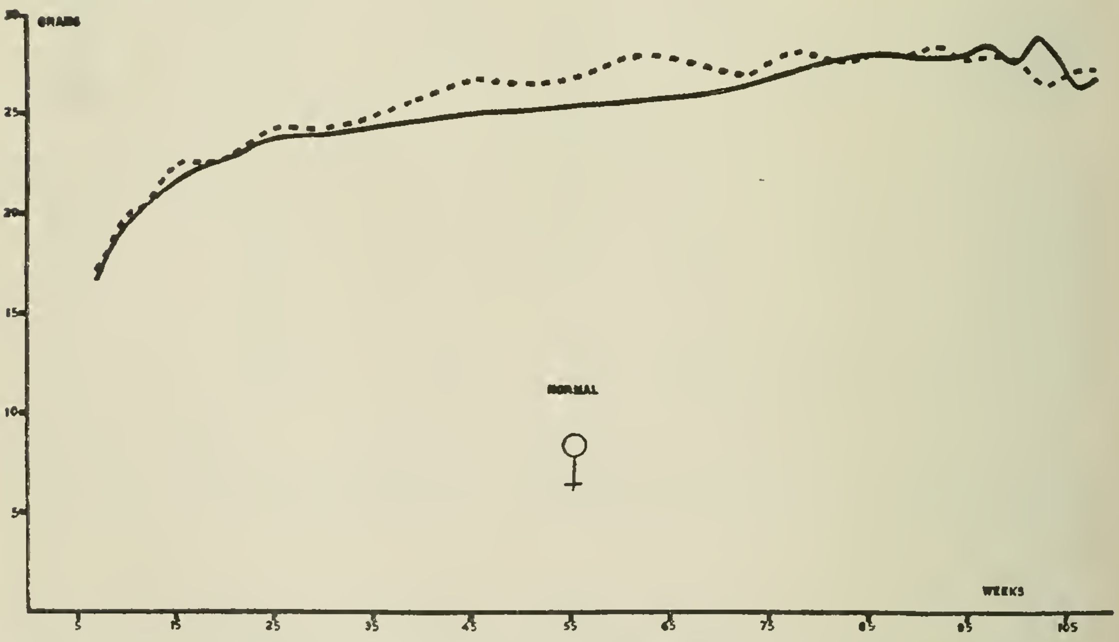

FIG. 2. COMPARISON OF THE GROWTH OF NORMAL FEMALE WHITE MICE WHICH ULTIMATELY DEVELOPED CARCINOMA (BROKEN LINE) WITH THAT OF NORMAL FEMALE WHITE MICE WHICH DID NOT DEVELOP CARCINOMA (FULL LINE)

or inferior in weight to the non-carcinomatous animals. The superior growth occurred during the period of developing sexual maturity (adolescent growth) and was merely maintained or slightly diminished or even lost during later life. These facts are summarized in table 2.

It will be seen that in four out of the eleven groups the animals which developed carcinoma were initially inferior in weight to the animals which did not subsequently develop carcinoma. Yet even in these cases superiority of weight was ultimately attained, and in three out of the four groups superiority of weight was attained before the thirtieth week. In the remaining group (lecithin-fed females) the initial inferiority was very great (1.65 grams, or 15 per cent of the mean weight) yet this group attained equality to the non-carcinomatous animals before the thirtieth week, and surpassed them thereafter.

TABLE 2

Relative weights at different ages of animals which developed carcinoma and those which did not. + indicates superiority of the animals which developed cancer; − indicates their inferiority in weight, and = indicates substantial equality of the two groups

CLASS OF ANIMALS	INITIAL WEIGHT (4 TO 5 WEEKS	10 TO 30 WEEKS	30 TO 70 WEEKS	70 WEEKS TO DEATH OF ONE HALF OF ANIMALS WHICH DEVELOPED CARCINOMA
Males.				
Normal	=	+	=	=
Pituitary	=	+	−	−
Lecithin	+	+	+	=
Cholesterol	+	+	+	+
Tethelin	=	+	+	+
Females				
Normal	=	+	+	=
Pituitary	−	+	+	+
Lecithin	−	=	+	+
Cholesterol	−	+	+	+
Tethelin (discontinuous administration)	−	+	+	−
Tethelin (brief administration)	+	+	+	+

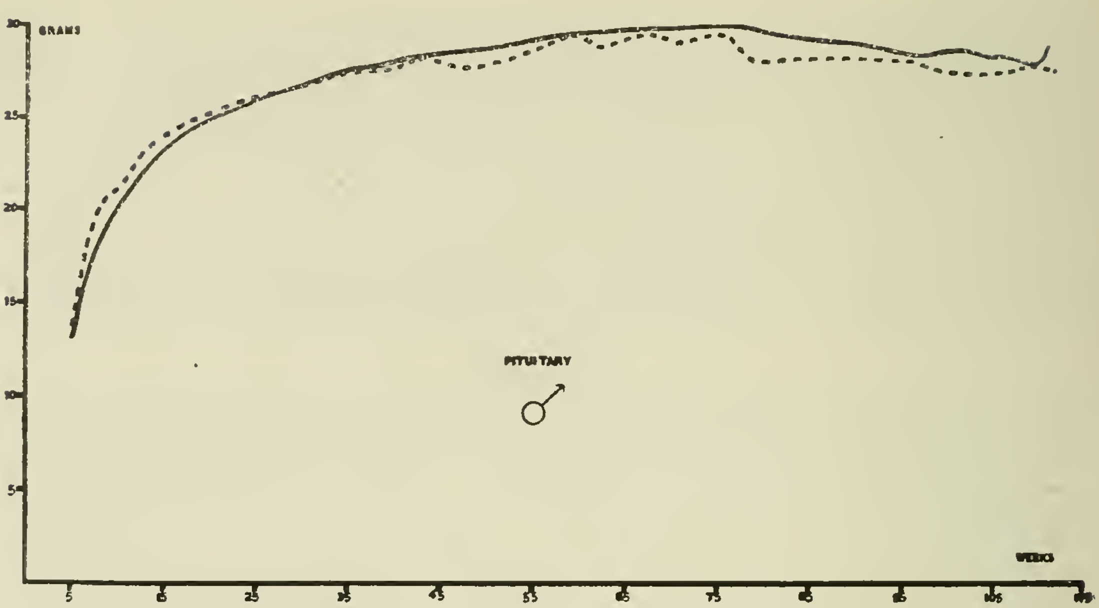

Fig. 3. Comparison of the Growth of Pituitary Fed Male White Mice Which Ultimately Developed Carcinoma (Broken Line) with that of Pituitary Fed Male White Mice Which Did Not Develop Carcinoma (Full Line)

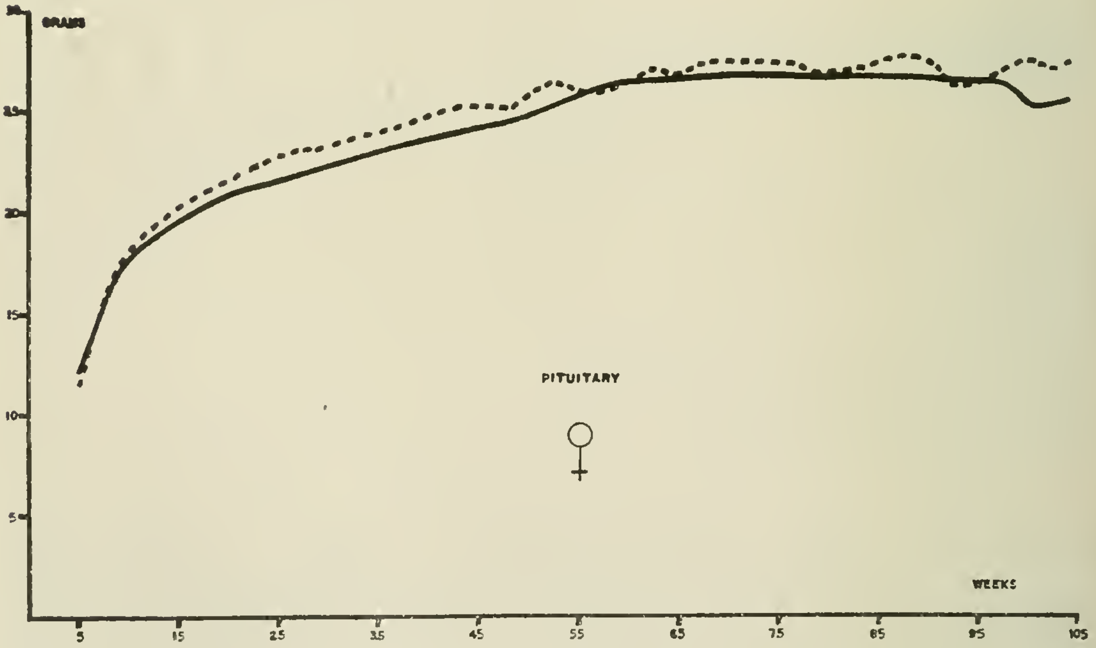

Fig. 4. Comparison of the Growth of Pituitary Fed Female White Mice Which Ultimately Developed Carcinoma (Broken Line) with that of Pituitary Fed Female White Mice Which Did Not Develop Carcinoma (Full Line)

INTERPRETATION OF THE RESULTS

We may infer that the animals which ultimately develop carcinoma foreshadow this outcome in their development during the earlier portions of the third or adolescent growth-cycle. They are distinguished from the animals which do not subsequently develop carcinoma by their exceptionally energetic growth during the period of adolescence. Prior to this period they do not display any uniform characteristics of weight, and subsequently to the thirtieth week the growth curve of the carcinomatous animals usually remains parallel to the curve for the non-carcinomatous animals or approaches it, the only marked exception to this rule being afforded by the group of cholesterol-fed females in which the animals which subsequently developed carcinoma display a late accretion of weight between the forty-fifth and the sixty-fifth week.

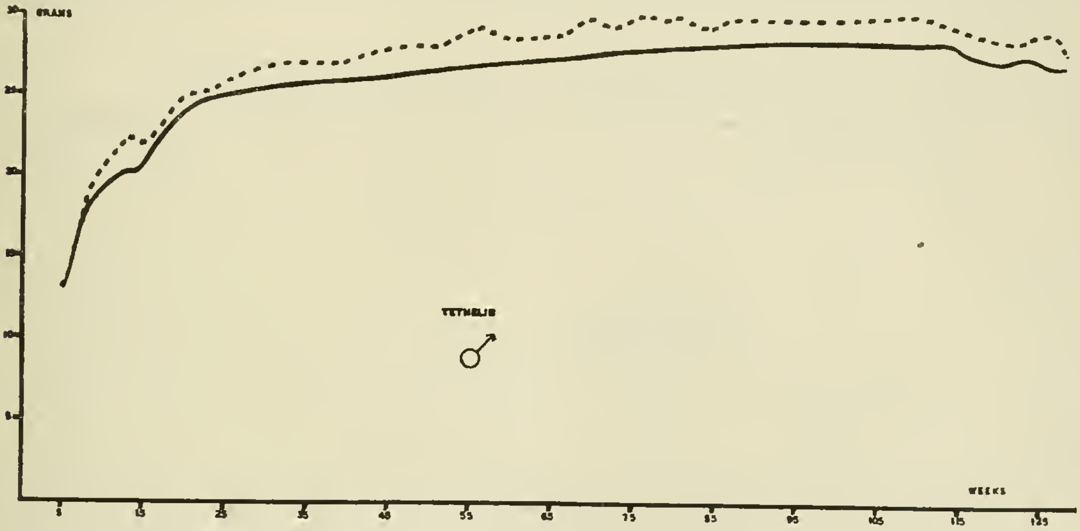

Fig. 5. Comparison of the Growth of Tethelin Fed Male White Mice Which Ultimately Developed Carcinoma (Broken Line) with that of Tethelin Fed Male White Mice Which Did Not Develop Carcinoma (Full Line)

The same data have afforded material for another significant comparison, namely, that of the growth of animals which survived beyond the average duration of life in any given experimental group with the growth of the animals which failed to attain the average duration of life (2). In this comparison it was shown that the long-lived animals are distinguished by relatively energetic early growth, low variability, steadiness of weight, and absence of late accretions of weight. The short-lived animals, on the contrary, display relatively deficient early growth, high variability, instability of weight, and a marked tendency to acquire late accretions of weight.

The animals which ultimately develop carcinoma, therefore, display the general characteristics of the long-lived groups of animals. At first sight this is not surprising, for the average duration of life of the animals which develop carcinoma exceeds, in eight of the eleven experimental groups, the average life-duration of the group. On closer examination, however, this result is not so readily intelligible as it appears. In the first place, the carcinomatous animals display the characteristics of the long-lived animals to an exaggerated degree, and, in the second place, only a little over two-thirds of the carcinomatous animals actually belonged to the long-lived group (table 3). On the other hand, of course, many long-lived animals and, in fact, most of those which survived for the longest periods, failed to develop carcinoma at all.

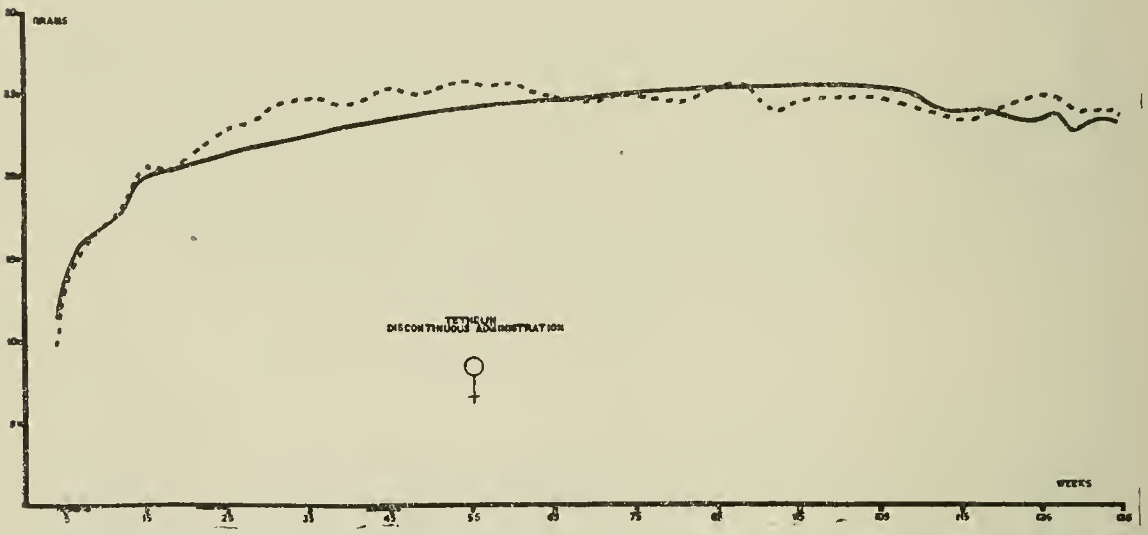

Fig. 6. Comparison of the Growth of Tethelin Fed Female White Mice (Discontinuous Administration) Which Ultimately Developed Carcinoma (Broken Line) with that of Tethelin Fed Female White Mice (Discontinuous Administration) Which Did Not Develop Carcinoma (Full Line)

We cannot, therefore, conclude that these results are due simply to the longevity induced by the superior growth-energy of the carcinomatous animals passively carrying them into the age-zone in which carcinoma occurs. A majority of all the animals attain the ages at which one-third of the carcinomatous animals die. A small minority of animals exceed the age at which the last carcinomatous animal has died, without developing carcinoma at all. Moreover, while the lowest incidence of carcinoma recorded in table 1 occurs in the shortest-lived group (tethelin, "brief administration") yet the highest incidence occurs in a group which lived an average of but twenty-four days longer (pituitary-fed females), and the next lowest incidence occurs in the longest-lived group of females (tethelin, "discontinuous administration").

TABLE 3

Showing the proportion of animals which developed carcinoma which also lived for longer than the average duration of life

CLASS OF ANIMALS	TOTAL NUMBER OF ANIMALS	NUMBER OF ANIMALS WHICH DEVELOPED CARCINOMA	NUMBER OF ANIMALS DEVELOPING CARCINOMA WHICH ALSO WERE LONG LIVED
Males			
Normal	32	8	6
Pituitary	31	9	7
Lecithin	32	7	5
Cholesterol	34	8	7
Tethelin	23	8	7
Totals	152	40	32
Females			
Normal	32	12	8
Pituitary	35	18	11
Lecithin	34	11	6
Cholesterol	32	16	9
Tethelin (discontinuous administration)	16	4	4
Tethelin (brief administration)	23	4	2
Totals	172	65	40

A remarkable feature of the results is their uniformity. This becomes the more noteworthy when we consider the variability of the experimental material. No estimates of variability in quantitative terms have been computed, because in many cases the small number of animals which ultimately developed carcinoma in any one experimental group rendered such a computation of very little value. Nevertheless, it may readily be perceived that there is no single type to which the carcinomatous animal adheres. No such simple rule can be formulated as that heavy animals develop carcinoma. Thus in the tethelin ("brief administration") group one of the animals which developed carcinoma weighed but 22.5 grams at seventy weeks, or nearly 7.5 grams less than the average weight at the same age of the non-carcinomatous animals of the same experimental group. Similar phenomena were observed in the other experimental groups. For this reason, of course, it is impossible to foretell the incidence

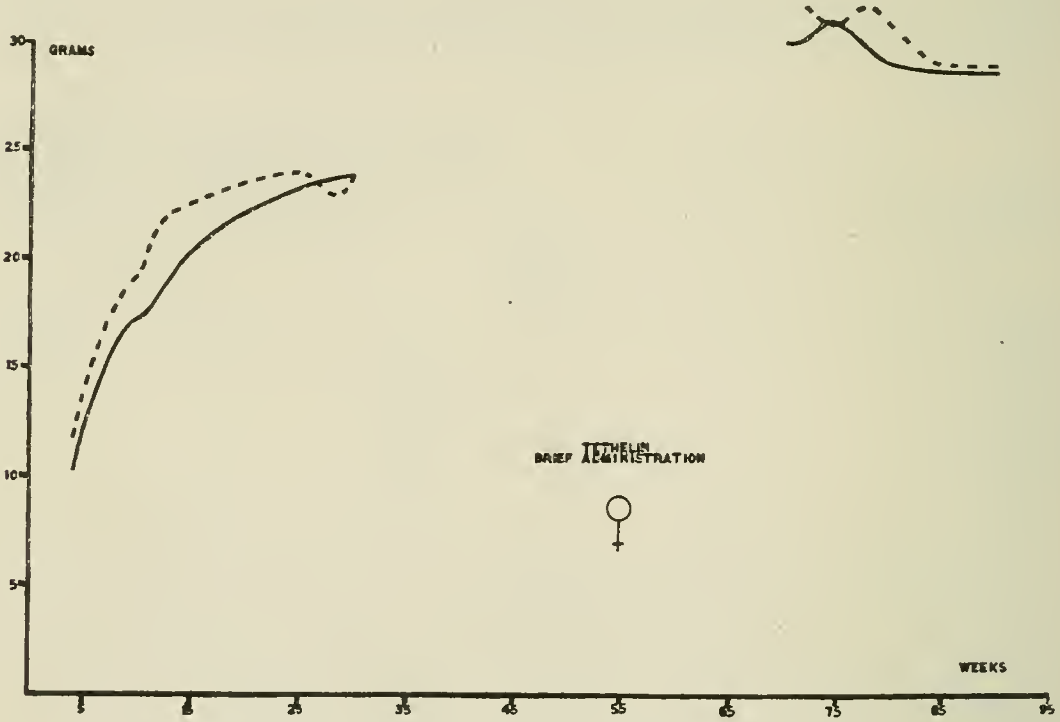

Fig. 7. Comparison of the Growth of Tethelin Fed Female White Mice (Brief Administration) Which Ultimately Developed Carcinoma (Broken Line) with that of Tethelin Fed Female White Mice (Brief Administration) Which Did Not Develop Carcinoma (Full Line)

of carcinoma in any individual animal from its preceding growth. How then does it arise that the average weight of the animals which subsequently develop carcinoma is so invariably in excess of the average weight of non-carcinomatous animals throughout a large proportion of their lives?

There would appear to be only one possible explanation, namely that it is *superiority of growth relatively to the standard norm of the strain* which foreshadows carcinoma, not energetic growth *per se*. The animals employed in this, as in any other animal experiment, represented a variety of more or less widely differing strains, each possessed of its own standard of absolute and relative development. In any sufficiently large group of animals chosen at random from the general stock, each of the main strains would be represented in proportion to its frequency. Since the carcinomatous animals, although so variable among themselves, invariably displayed an *average* weight which for a large period of their lives exceeded the *average* weight of the remainder, we must conclude that each of the various strains

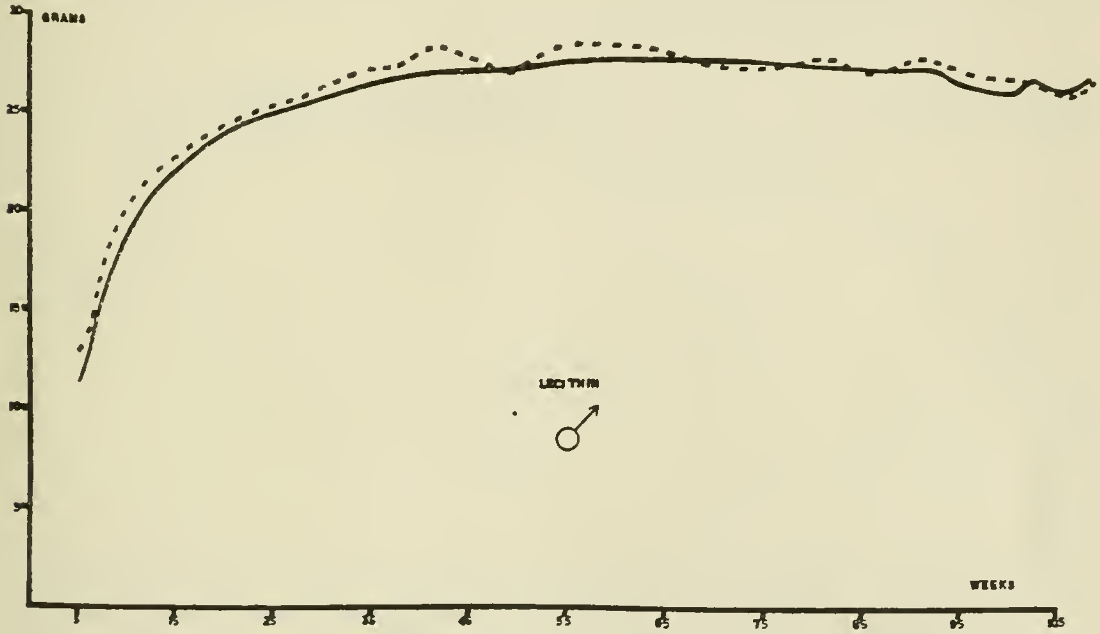

FIG. 8. COMPARISON OF THE GROWTH OF LECITHIN FED MALE WHITE MICE WHICH ULTIMATELY DEVELOPED CARCINOMA (BROKEN LINE) WITH THAT OF LECITHIN FED MALE WHITE MICE WHICH DID NOT DEVELOP CARCINOMA (FULL LINE)

in the stock furnished its proportionate quota to the carcinomatous group, and in each strain the animals which ultimately developed carcinoma were superior in energy of growth to the standard norm of the strain. This fact precludes the idea that we may be dealing with linked size and carcinoma-inheritance, the carcinomatous animals representing a single strain or a small number of strains distinguished also by large size. The two factors, that of absolute size and that of tendency to develop carcinoma, are, on the contrary, separable.

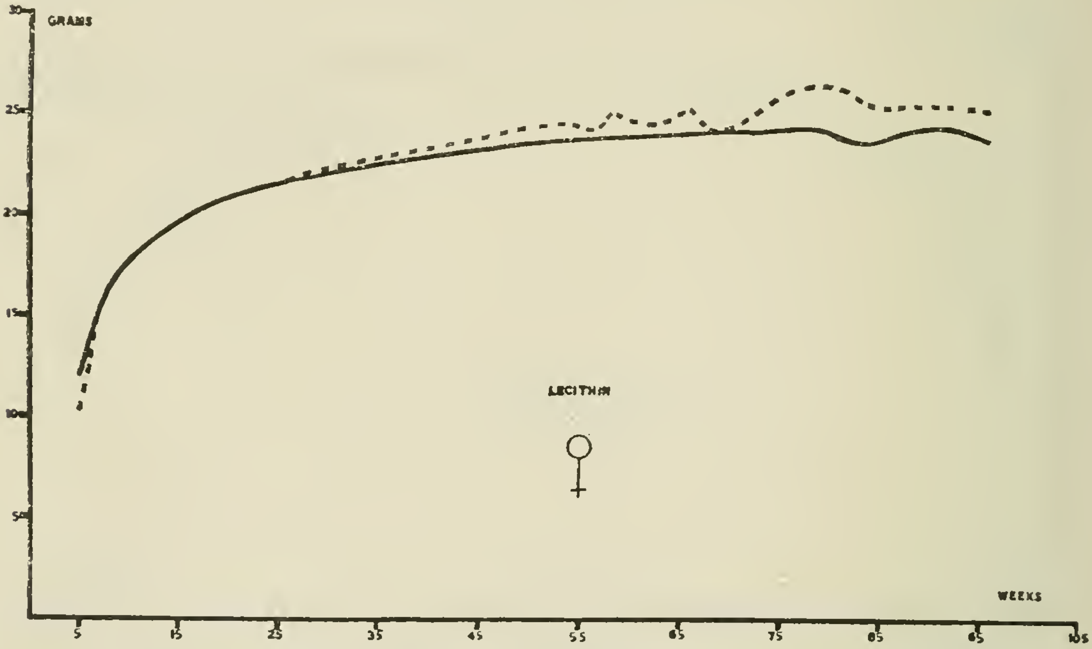

FIG. 9. COMPARISON OF THE GROWTH OF LECITHIN FED FEMALE WHITE MICE WHICH ULTIMATELY DEVELOPED CARCINOMA (BROKEN LINE) WITH THAT OF LECITHIN FED FEMALE WHITE MICE WHICH DID NOT DEVELOP CARCINOMA (FULL LINE)

In the article dealing with the comparison of the growth of long-lived with that of short-lived animals, it was sought to interpret the results in terms of the competition between parenchymatous and connective tissues during the development of the animals. In those animals in which the anabolism of parenchymatous tissues is exceptionally rapid, the growth of parenchymatous structures will be hastened and facilitated. This may or may not tend to increased weight of the animal at the time, according to the degree of acceleration experienced and the relative masses of parenchyma and connective tissues in the body of the animal. It will, however, manifestly delay, through previous appropriation of foodstuffs, the senescent accretion of connective tissues, and consequently increase the duration of life.

If we apply this hypothesis to the results enumerated in this paper we must conclude that the animals which subsequently develop carcinoma are distinguished by exceptionally rapid anabolism and growth of parenchyma which, during adolescence, more than compensates for the complementary delay in connective tissue growth. In the absence of carcinoma this would result in great longevity, which however, is curtailed by a pathological proliferation of parenchymatous tissue. We may suppose that in such animals the energy of response to some external stimulus, such as a local source of irritation, is so excessive that when the age of the animal is sufficiently advanced to have resulted in some weakening of the competition of other parenchyma (through the parasitism of connective tissues) the effect is to institute a growth which appropriates nutriment to itself at the expense of all the adjacent tissues.

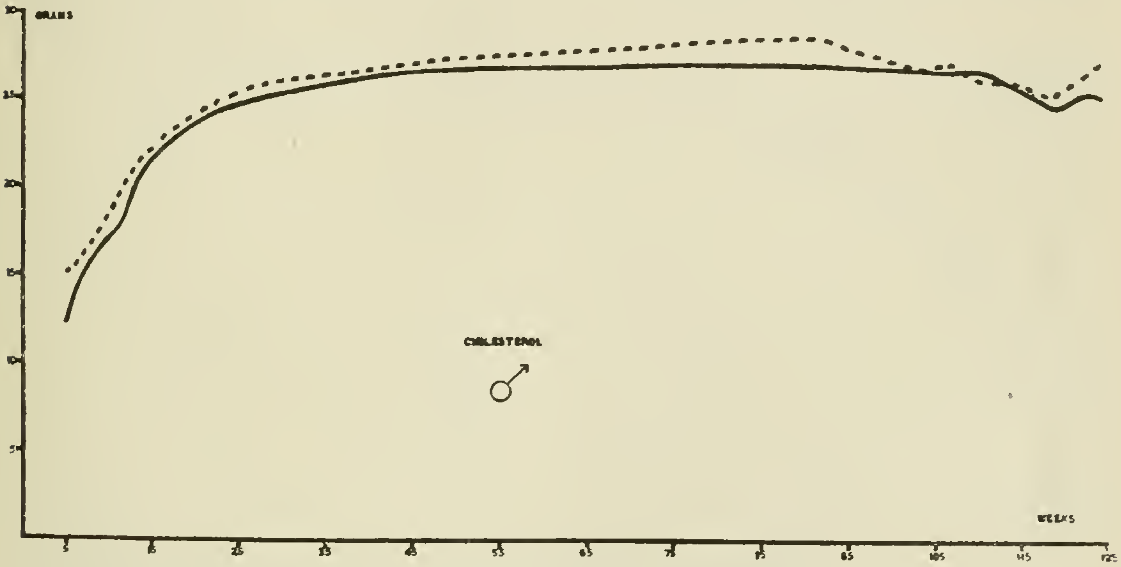

FIG. 10. COMPARISON OF THE GROWTH OF CHOLESTEROL FED MALE WHITE MICE WHICH ULTIMATELY DEVELOPED CARCINOMA (BROKEN LINE) WITH THAT OF CHOLESTEROL FED MALE WHITE MICE WHICH DID NOT DEVELOP CARCINOMA (FULL LINE)

In accordance with this hypothesis we find that the incidence of carcinoma is lowest in that experimental group in which the growth curve departs most widely from the long-lived type, namely, in the tethelin "brief administration" group in which there is an extraordinary late accretion of tissue (3). The only other group of carcinomatous animals which reveals late accretion of weight is also the group which has the briefest duration of life (cholesterol-fed females, table 1).

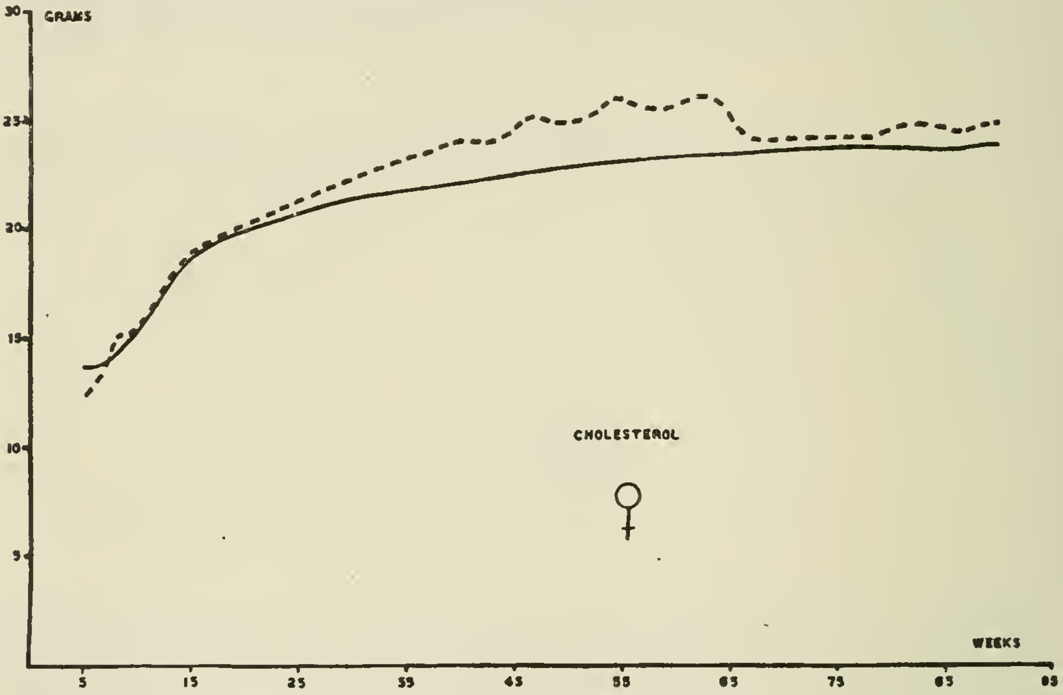

FIG. 11. COMPARISON OF THE GROWTH OF CHOLESTEROL FED FEMALE WHITE MICE WHICH ULTIMATELY DEVELOPED CARCINOMA (BROKEN LINE) WITH THAT OF CHOLESTEROL FED FEMALE WHITE MICE WHICH DID NOT DEVELOP CARCINOMA (FULL LINE)

The longest-lived animals in any group must, therefore, be those in which the speed of anabolism and energy of growth of the parenchyma just fall short of the tendency to respond abnormally, by the formation of neoplasms, to recurring local irritative stimuli.

The comparative growth of animals which ultimately developed neoplasms other than carcinoma will form the subject of subsequent communications.

SUMMARY

1. The incidence of carcinoma in mice is foreshadowed in their preceding development; but, owing to the variability of the animals, it is not possible to foretell the incidence of carcinoma in any single animal from its curve of growth.

2. The animals which ultimately develop carcinoma are distinguished by relatively energetic growth during the period of adolescence. The lead over the other animals which is thus established is usually maintained throughout life, but is sometimes lost in the later periods of life through late accretion of weight by the animals which do not develop carcinoma.

3. These results do not admit of interpretation by supposing that superior growth favors longevity, and thus passively carries the animals into the age-zone in which carcinoma occurs. A majority of all the animals surpass at death the ages at which one-third of the carcinomatous animals die. There is no correlation between the percentage incidence of cancer in any group and its average duration of life; but animals displaying an average growth curve which departs widely from the carcinomatous type also display a low percentage incidence of carcinoma.

4. The results are not due to linked inheritance of size and tendency to develop carcinoma, since the factors of absolute size and development of carcinoma are separable.

5. The results are interpreted to mean that the animals which ultimately develop carcinoma are those in which the anabolism and, therefore, the growth of parenchyma is exceptionally rapid. In such animals the energy of response to local irritative stimuli may be so excessive as to overcome the competition of other tissues and initiate a new growth.

6. The longest-lived animals in any group are, therefore, those in which the speed of anabolism and energy of growth of the parenchyma just fall short of those which lead, under the influence of recurrent irritative stimuli, to the formation of new growths.

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EFFECT OF BLOOD FROM IMMUNE ANIMALS UPON TRANSPLANTABLE TUMORS

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From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director.

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Even a cursory review of research in experimental cancer will reveal many inconsistencies, various workers having obtained contradictory results when attacking a problem with apparently similar methods. In many cases, however, patient repetition with careful checking up of the findings has brought to light factors which offer a satisfactory explanation for these puzzling variations. In this study an attempt was made to clear up one of the problems in which different workers have reached diametrically opposed conclusions.

Jensen (1) reported the disappearance of transplanted tumors in mice injected with serum from rabbits treated with carcinoma. In a later publication (2) he stated that while he realized that spontaneous retrogression would explain most of his "cures," it did not account for the disappearance of several of the very large tumors.

von Leyden and Blumenthal (3) reported the successful outcome of the treatment of a dog with a carcinoma, the diagnosis of the growth having been verified by microscopical examination. The method was as follows: Several rabbits were given a series of subcutaneous injections of carcinoma emulsion over a period of several weeks, and the serum obtained from these rabbits was injected subcutaneously into the dog.

Clowes (4) reported the results of experiments with mice in which one half of the animals received injections of "immune" serum obtained from mice whose tumors had retrogressed spontaneously. The other half acted as controls and received normal mouse serum. Of the twenty mice in the first series, only one failed to show the beneficial effect of the immune serum, and all were alive at the time of the report; while in the control series, five were dead and all the others had tumors exceeding in size those of the first series. Clowes and Baeslack (5) reported the results of experiments in which one lot of mice was treated with tumor mixed with immune serum, a second with tumor mixed with normal serum, and a third with tumor mixed with normal saline solution. The difference between the results obtained in the two latter groups was negligible. The difference between the results in the "immune" set and in the two control sets, however, was considerable, 31.6 per cent of tumors developing in the controls and only 12.3 per cent in the immune group. On the basis of these findings, they justified their assumption of the presence in the blood of immune bodies antagonistic to the development of carcinoma.

Crile and Beebe (6) reported a series of ten blood transfusions in dogs with an infectious lymphosarcoma, resulting in cure in seven cases and in marked improvement in two. In the remaining case, in which the treatment was a complete failure, it was later found that the donor was not an immune animal.

von Dungern (7) obtained serum from rabbits in which there has occurred spontaneous retrogression of a sarcoma. Seven rabbits were treated by intravenous injection of this serum before being inoculated with sarcoma. Nine other rabbits were employed as controls. Six of the control animals developed tumors; in none of the treated animals did a tumor develop. Uhlenhuth, Händel, and Steffenhagen (8), however, performed the same experiment and found that rats treated with "immune" serum gave 94 per cent of successful takes, whereas those treated with normal serum gave 83 per cent, and those that received no treatment at all gave only 66 per cent. In another series of experiments, these authors mixed an emulsion of tumor tissue with immune serum. The injection of this mixture gave 100 per cent successful takes. In their experience heterologous immune serum proved just as unsuccessful as the homologous serum.

Haaland (9) treated Berlin mice with the serum of immune Hamburg mice. On subsequent inoculation of the Berlin mice with a sarcoma to which they were normally susceptible the tumors grew just as well as they did in the untreated controls. He then repeated the experiments, employing the serum from immune Danish mice; but here again there was no difference between the growth of the tumors in treated and in untreated animals.

Russell (10) and Bashford, Murray, and Cramer (11) also failed to obtain positive results with the blood from immune animals.

Sisto (12) repeated the experiments of von Dungern (13) and others in attempting to influence tumor growth by the injection of splenotoxic, orchidotoxic, and hepatotoxic sera from rabbits into which emulsions of the fresh respective rabbit organ had been injected. His published charts show practically no effect of the injections one way or the other.

It would not be amiss in a paper of this character to make mention of a closely allied but not quite similar method of treatment that has been tried in man. In 1910 Hodenpyl (14) published a preliminary report of the treatment of carcinoma by the subcutaneous and intravenous injection of ascitic fluid from a patient who, he believed, had undergone a spontaneous recovery from carcinoma. All of his patients showed subjective improvement. It is, however, well known to those who have had experience in the treatment of inoperable carcinoma that every new form of therapy along this line is followed by a temporary subjective improvement, i.e., relief of pain, diminution of weakness, improvement in appetite, etc., only to be succeeded by the inevitably fatal termination. Hodenpyl stated that the tumors diminished in size and that some disappeared. No cures were obtained, however, and the donor of the ascitic fluid also died later from cancer.

Ill and Miningham (15) repeated Hodenpyl's treatment in a series of twenty-seven cases in which ascitic fluid was injected subcutaneously. The fluid was obtained from a patient who was apparently recovering from carcinoma of the liver; but autopsy later on revealed widespread carcinoma of the liver, ovaries, and intestines. The authors noted marked subjective improvement, although they too failed to produce a single cure with this treatment.

In the experiments about to be reported, the procedure was as follows: The rats used were those known in the laboratory as the Marshall and the August breeds. The Marshall rats are susceptible to the Flexner rat carcinoma and refractory to the Jensen rat sarcoma. The August rats have the reverse characteristics in respect to these two tumors. To make doubly sure that the animals used were immune, a preliminary inoculation of the Flexner rat carcinoma (FRC) was made in the August, and of the Jensen rat sarcoma (JRS) in the Marshall rats. Only those animals were used which did not develop tumors or in which there was a small growth with subsequent complete retrogression. These animals constituted the stock from which the immune blood was taken.

One group of Marshall rats was treated by intraperitoneal injections of blood from the August rats which had showed a natural immunity against the Flexner rat carcinoma. A second group of Marshall rats received an intraperitoneal injection of blood from normal Marshall rats. A third group received no preliminary injection of blood. All three groups were then inoculated with grafts of 0.002 gram of the FRC in the right axilla. Exactly the same plan was followed with the August rats, the immune Marshall rats being used as the source for the immune blood, and the JRS being inoculated. The amount of blood injected varied from 2 to 3 cc. depending upon the size of the animal, the idea being to take as much blood as possible without killing the rat. The tumor inoculation was made synchronously with the blood injection or within a period of forty-eight hours after it.

In accordance with the theory of the workers cited, there should have been a complete absence of growth or at least a noticeable retardation of growth in those animals that had been treated with blood taken from the immune animals. Examination of the records, however, showed that the rats treated with the immune blood not only did not show any absence or retardation of growth of the tumor, but actually showed tumors that were in most instances larger, and developed earlier than those in the untreated animals. Even the animals treated with normal blood showed this phenomenon though in a lesser degree. Gay (16) found that injection of blood from insusceptible or refractory animals lead to an increase in the number of takes of carcinoma implantation.

Another series of experiments was made in order to determine whether the blood or other proteins injected act as a food. Three sets of rats, 36 in each group, were employed. One group received 1 cc. of blood (18 rats subcutaneously, and 18 intraperitoneally) every three days over a period of six weeks. A second group received 1 cc. of egg-white (18 subcutaneously, and 18 intraperitoneally) every three days over a similar period. A third group was kept as controls. All these animals were inoculated with the JRS, and the tumors were charted every week for eight weeks. No appreciable difference was noted in the growth of the tumors in the three groups. Hence, the possibility that the injections acted as nutriment could be disregarded.

CONCLUSIONS

It is apparent from these experiments that:

1. If immune bodies do exist in animals that are refractory to tumor growth, they are not resident in the circulating blood.
2. The transfusion of blood, if it has any influence, accelerates the development of a tumor, as regards both time and intensity of growth; the inadvisability of transfusing human cancer cases is therefore evident.
3. The results obtained by those investigators who report successful cures after injections of blood or serum must be explained by the assumption that they were dealing either with infectious granulomata or with tumors that disappeared spontaneously.

In order to reduce the effects of extraneous factors as much as possible, the tests were conducted in eight successive series, each set containing five test animals and ten controls. The test animals were those treated with preliminary injections of "immune" blood, the controls those that received injections of normal blood or nothing at all.

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INOCULATION OF SARCOMATOUS TUMORS INTO NEGRO FOWLS, WITH SPECIAL REFERENCE TO THE SIGNIFICANCE OF CHROMATOPHORES

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A kind of domestic fowl, called in western countries "silky fowl" or "negro fowl," has its origin in the Orient. Their most striking characteristics are, first, the extra toes on the inner side of the foot, showing a complete or incomplete adherence of first and second of them, and second, marked pigmentation of every part of the body. Although the feathers of genuine negro fowls are pure white or pure black, the skin itself always appears extremely bluish brown in color and contains a number of chromatophores in the cutaneous and subcutaneous connective tissue. The periosteum and endosteum, dura and pia mater, perivascular connective tissue and serosa also show the brown coloration, diffuse and spotted. Of the organs the testicles and ovaries are most strongly tinted; the lungs show spotted pigmentation as in anthracosis. The parenchymatous organs, namely, the liver, kidney, and spleen, are usually free from abnormal pigmentation. The coloration of bone is so remarkable that we suspected at first its identity with animal ochronosis.

Microscopically we ascertain that the brown coloration everywhere in the body is due to the existence of a large number of chromatophores in the interstitial tissue, especially along the course of the blood-vessels. The chromatophores assume a form quite similar to those in normal fowl, having a number of irregular long and slender processes and fine yellowish brown pigment granules with an entire similarity to melanin in microscopical appearance. Pigment granules in the cells which are in resting stage are very fine and equal in size. When stimulated by experimental means they become irregular in size, and the cells lose their processes, taking a round shape like any other plastic cells.

The brown coloration of the bone is caused by the pigment cells in the periosteum. The endosteum of the wing bone, which covers the inner surface of empty medullary cavity, has also a number of chromatophores. The matrix of the bone is free from any kind of pigment, in contradistinction to the condition found in animal ochronosis, while the bone-corpuscles contain genuine melanin granules in the protoplasm, which cause a slight brown coloration of the bone tissue. This is especially evident in internal and external layers, near the peri- and endosteum. The bone marrow also contains chromatophores in greater or less number.

The cartilage shows generally no marked coloration. The cartilage cells, however, sometimes contain microscopically a number of pigment granules. The dura mater is deep brown like the periosteum. The pia mater shows grayish brown spots due to pigment cells. The fascia is more or less brown in color, owing to a number of chromatophores which are sometimes to be seen in intermuscular connective tissue. The periosteum, pleura, and synovial membrane also show spotted pigmentation. The pigmentation of the testicles and ovaries is caused by the existence of chromatophores in the interstitial tissue, while the tubules and the follicles have no pigment particles. A few pigment cells are found in the capsules of the spleen, liver, kidney, and pancreas, and in the perivascular connective tissue. The parenchyma of the organs and the stellate cells in the liver contain no pigment granules.

The principal subject of our investigation with this kind of fowl was to determine whether or not the chromatophores take part in the neoplastic proliferation brought about by the experimental inoculation of transplantable tumors. Such participation, if found to occur, would be undoubtedly reliable evidence of the secondary acquirement of the neoplastic properties by normal tissue. Another object was to ascertain the origin of melanotic pigment in bone and cartilage cells in negro fowls, that is, whether it is formed in osteoblastic and chondroblastic cells themselves or is given to them by adjacent chromatophores. Moreover, we expected this material to be most suitable for the purpose of studying the relation between the pigment in epithelial cells and chromatophores, which has been an interesting subject of discussion among authors.

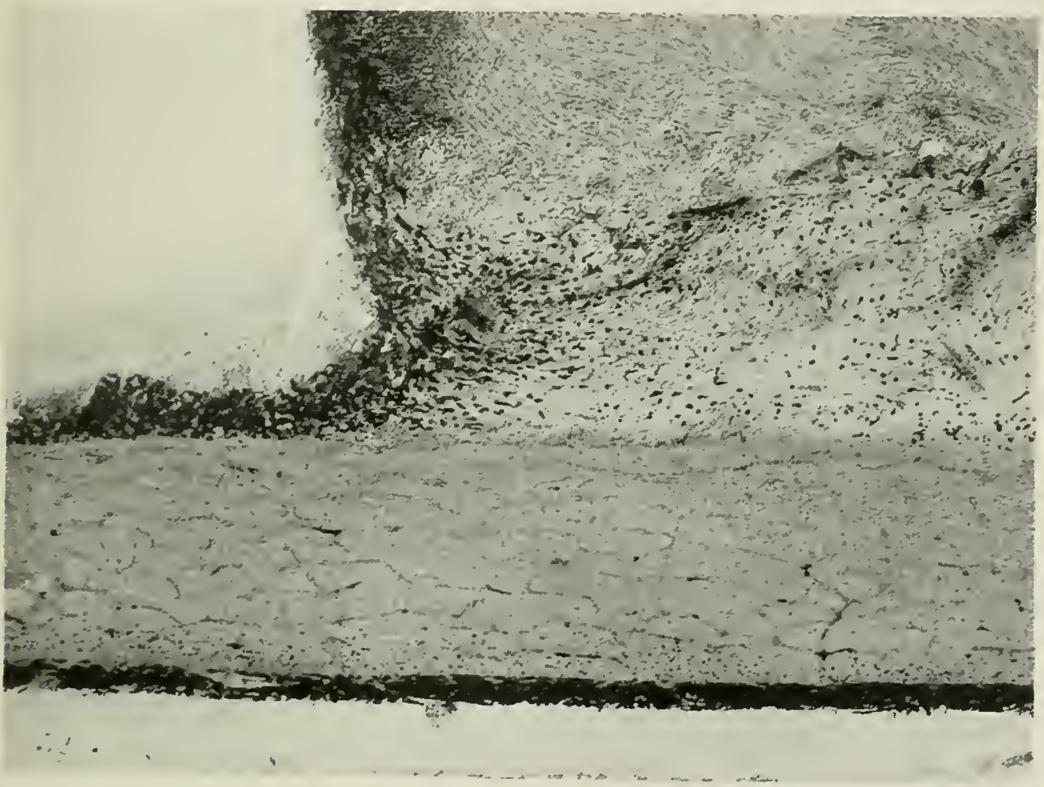

FIG. 1. Beginning myxosarcomatous growth in the endosteum of the wing bone of a negro fowl, showing a number of chromatophores in the periosteum and endosteum. The chromatophores in the endosteum are deranged and replaced by the new grown tissue. Bone corpuscles contain melanin granules, especially evident near the periosteum.

I. THE INOCULATION OF TRANSPLANTABLE TUMORS INTO NEGRO FOWLS

Two different strains of transplantable tumors (chondrosarcoma and myxosarcoma) were chosen for this purpose. Fifty negro fowls were inoculated, 15 of them with chondrosarcoma and 30 with myxosarcoma. We usually utilized for inoculation the empty medullary cavity of the wing bones, because the endosteum is rich in chromatophores and if any proliferation should take place it could be easily followed. The wing bone was exposed, perforated by means of a borer, and inoculated with the tumor. In the case of chondrosarcoma a small piece of tissue was used; in that of myxosarcoma, except in a few instances, 2 to 4 per cent watery filtrate of the tumor extract. The tumors were easily transplantable in the medullary cavity of the wing bone. Positive transplantations were obtained in 9 out of 15 inoculations with chondrosarcoma and nearly in all with myxosarcoma.

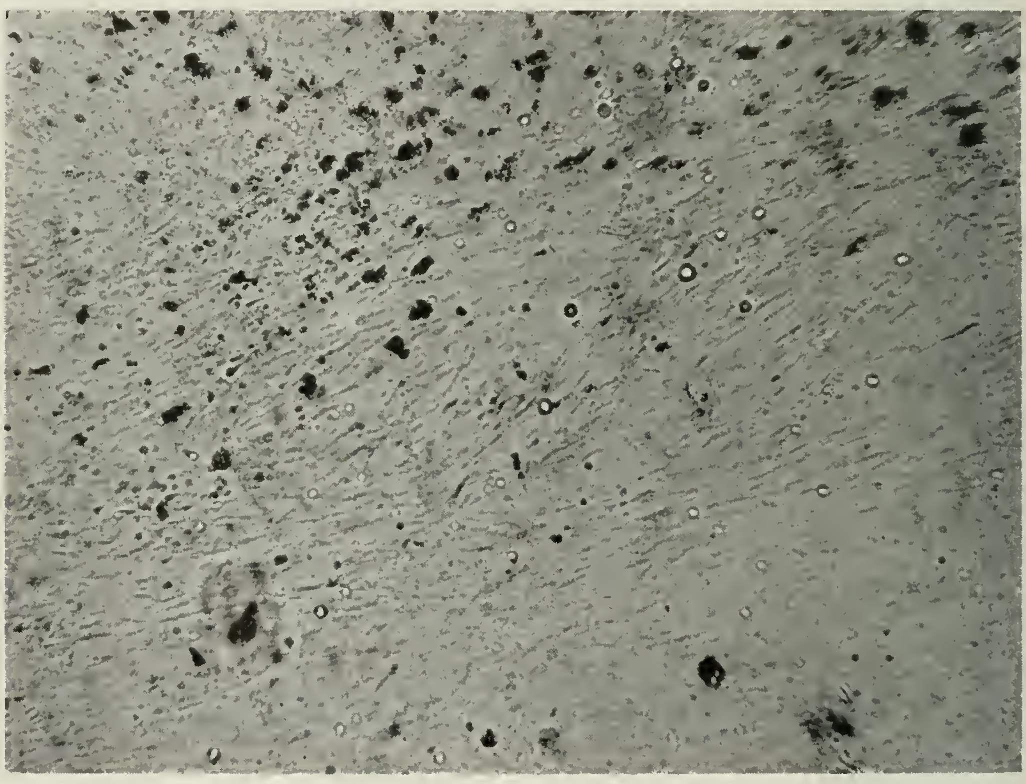

FIG. 2. Advanced myxosarcomatous tumor of a negro fowl, showing a number of melanotic tumor cells.

The development of chondrosarcoma results in a very great reactive formation of callus which consists of connective, cartilage, osteoid, and bone tissues. The neoplastic tissue, however, differs from the reactive callus tissue which is accompanied by a number of chromatophores. The chondromatous cells do not contain any particles of pigment. Briefly, in the series of inoculations of chondroma no neoplastic change of ordinary chromatophores was seen. On the contrary, the myxomatous tumor fills up the medullary cavity of the wing bone, accompanied by some reactive callus tissue. In our cases metastasis was frequently to be seen, especially multiple, into lungs and mediastinum, exceptionally into the skin, peritoneum, pericardium, and liver. The neoplastic tissue in the medullary cavities of wing bones is somewhat gray in color. Some of the metastatic tumors also show a grayish coloration.

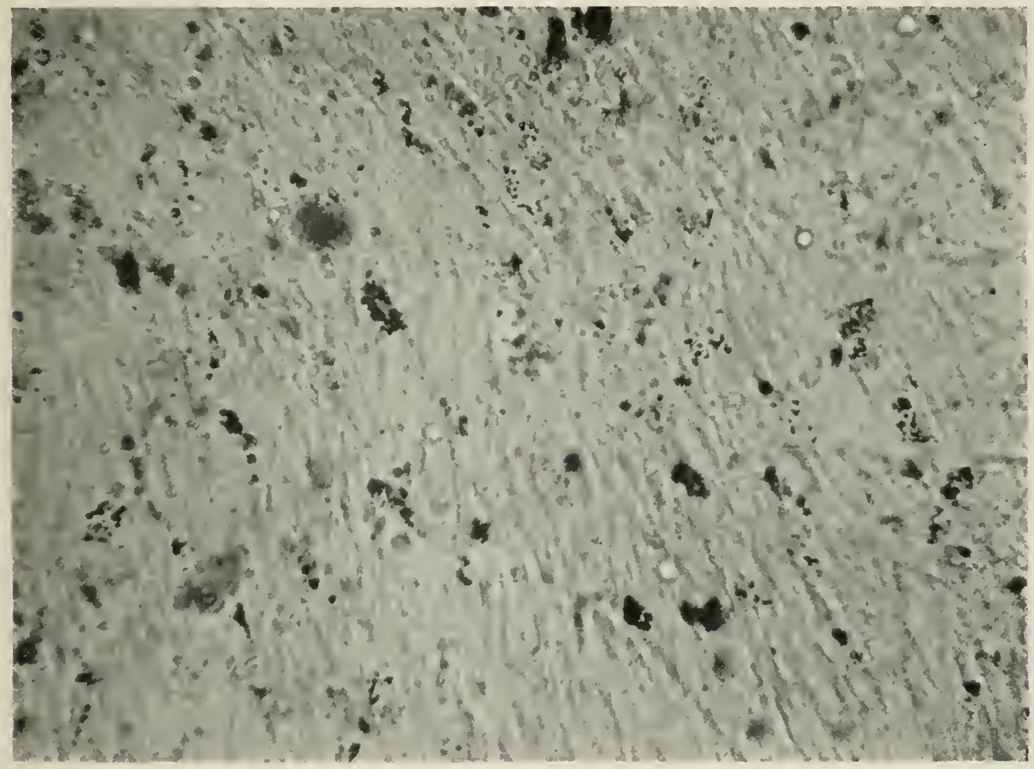

Fig. 3. The same specimen in higher enlargement.

Microscopically the neoplastic proliferation of endosteum in cases of inoculation with filtrate of tumor extract seemed to begin in the connective tissue in the layer close to the bone surface. The chromatophores in the endosteum are deranged and replaced by the new grown tissue. In this stage they did not show any sign of proliferation, having the usual form with a number of processes. Sooner or later, however, they began to multiply and produced numerous round cells with coarse pigment granules of irregular size. They were prolonged and transformed into long spindle cells which assumed an appearance quite similar to genuine tumor cells, not only in their form, but in their arrangement in the tumor tissue. Pigment granules are generally found close to the nucleus. Most of them, therefore, are to be considered as true neoplastic elements. The pigment may not be a general metabolic product of negro fowl, for the granules are found most abundantly near periosteum and endosteum, where chromatophores exist in large number. This finding confirms that of Rous, Fujinami, Hayashi and others, that the normal tissue may acquire neoplastic nature by the inoculation of the tumor tissue or its extract. The formation of a pure melanotic tumor is not to be expected, for the pigment cells are always accompanied by a large number of the usual connective tissue cells, which are more readily proliferative than the former. In further generations of inoculation of tumor tissue with melanotic elements into the normal fowls the pigmented tumor cells gradually disappear and the neoplastic proliferation of neighboring tissue cells overcomes the transplanted pigmented tumor cells.

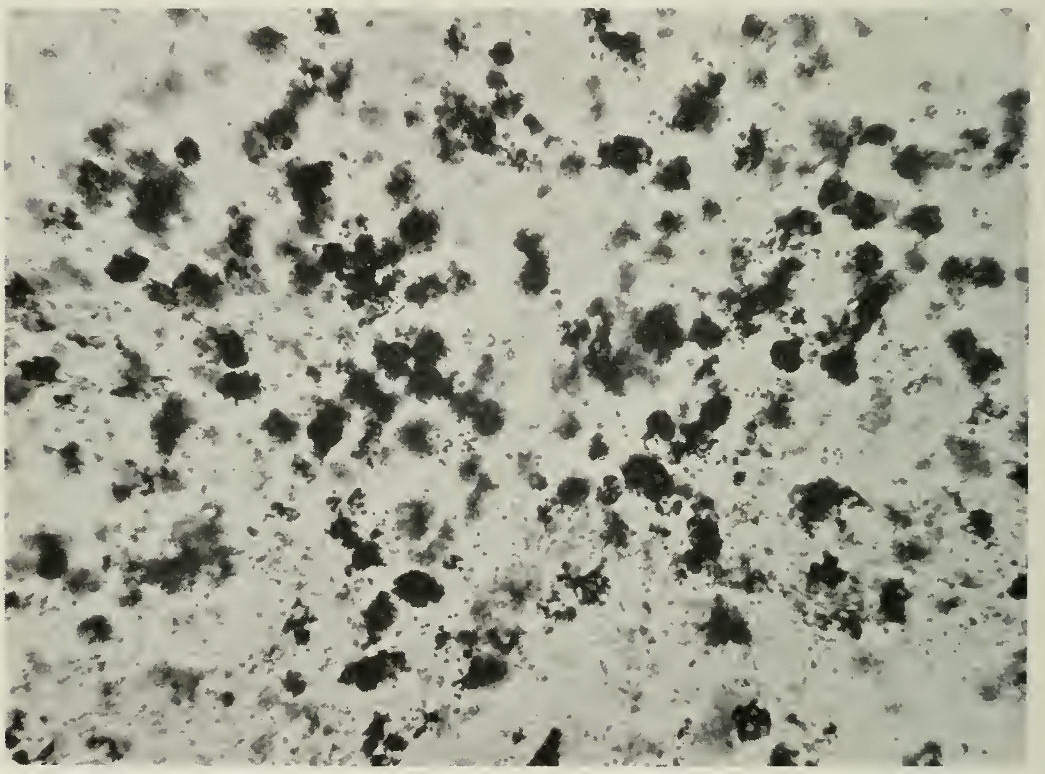

Fig. 4. Advanced myxosarcomatous tumor of a negro fowl with an enormous proliferation of melanotic cells.

II. THE RELATION BETWEEN THE MELANOTIC PIGMENT IN EPITHELIAL CELLS AND CHROMATOPHORES

With regard to the origin of the melanotic pigment in chromatophores and in epithelium a difference of opinion exists. Some authors maintain that the pigment originates in chromatophores and then goes over into epithelium, while others assert that it is primarily produced in epithelial cells. At the present time a majority of authors seem to consider that melanotic pigment is formed independently both in epithelium and in chromatophores.

The negro fowl offers a most suitable characteristic for the purpose of solving this problem. As mentioned, there are two kinds of negro fowl, white feathered and black feathered. Melanin granules are not to be found in the epithelium of the white feathered, while the black feathered variety has a number of pigment granules in the epithelial cells of the skin, more abundantly in the exposed parts such as the legs or the crest. In other parts of the body, where stratum corneum is thick, the melanin content of epithelium is generally light. The chromatophores in cutaneous and subcutaneus connective tissues differ in numbers among both kinds of negro fowls. In this point the white feathered negro fowl is to be compared with the ape (*Cercopithecus mona*), which has white hair and no pigment in the skin, but a large number of chromatophores in the cutaneous connective tissue. On the other hand the embryonal skin of the normal black feathered fowl shows the pigment only in the epithelial layer in the early stage. We recognized, therefore, that melanin pigment may be formed in both elements independently. The migration of the pigment granules from one to another seems to be disproved in this case, although the general idea that the pigment of the eye is formed in the retina and secondarily given to the cells of the choroid, may not be impossible.

III. THE ORIGIN OF MELANOTIC PIGMENT IN BONE CORPUSCLES AND IN CARTILAGE CELLS

It is rather important to determine whether the above mentioned pigment in bone and cartilage cells is formed in the cells themselves or is given from the adjacent chromatophores. For this purpose I have intentionally broken the ulna of negro fowls in one or both sides and examined the callus histologically in the course of one to forty-three days after fracture. Twelve negro fowls were used for this experiment. During healing of fractured bones the callus formation takes place at first close to the bone tissue, stripping off the layer of chromatophores. Periosteal chromatophores are arranged close to each other in the earlier stage. With the advance of callus formation they are separated by new grown tissue cells. Actual proliferation of chromatophores takes place in some degree, forming round shaped cells with irregular melanin granules. At the same time melanin granules are to be found in some typical osteoblasts and chondroblasts in the callus, but far less in number than in new formed chromatophores. Pigment granules in the plastic cells are generally coarse in size, but they do not show any microchemical difference from those in chromatophores. The amount of pigmented plastic cells is generally proportional to the number of chromatophores in the body. There is no actual sign of transference of pigment from chromatophores to plastic cells. The metaplastic change of chromatophores to fixed tissue cells is not recognizable, for the latter are to be acknowledged undoubtedly as a sort of wandering cell. We conclude, therefore, that genuine osteoblasts and chondroblasts themselves can make melanin into protoplasm, and that the melanin formation may occur in many kinds of tissue cells, if there are some melanogenetic compounds from which melanin could be derived, and fermentative (perhaps oxidative) elements which act on them.

SUMMARY

1. Oriental negro fowls have a large number of chromatophores in all parts of the body, especially in the connective tissue, dura

and pia mater, periosteum and endosteum, serous membrane, interstitial tissue of genital glands, and in the lungs.

2. The bone corpuscles and occasionally the cartilage cells also contain melanotic pigment in their protoplasm.

3. Myxosarcomatous tumors of negro fowls grown by inoculation of tissue or its extract assume a melanotic nature, probably due to the neoplastic proliferation of chromatophores. This is actual evidence for the acquisition by normal tissue of neoplastic nature.

4. The melanotic pigment in the epithelium and connective tissue is formed in both elements independently of each other. The migration of pigment granules from one to another is not recognizable.

5. Osteoblasts and chondroblasts in callus also form melanotic pigment in their protoplasma.

INFLUENCE OF THE LYMPHOCYTE ON THE PERITONEAL IMPLANTATION OF SARCOMA IN MICE

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No phase of tumor investigation in recent years has aroused greater interest than that of the relation of the lymphocyte to natural and induced resistance to cancer. Following the observation by Da Fano (1) that lymphoid and plasma cells are constantly associated with degenerating tumors and that the lymphocyte might be the agent which distributes immunity throughout the organism, many articles have been written tending to prove an antagonistic action between the lymphocyte and tumor growth. Burgess (2) noted that the retrogressing implant of cancer in mice is invariably surrounded by large numbers of lymphocytes. Similar observations were made by v. Dungern and Coca (3). Apolant (4) found increased difficulty in immunizing splenectomized animals to tumor implants. Tyzzer (5) working with a Japanese waltzing-mouse tumor found that implants grew vigorously for a time in non-susceptible mice, but were destroyed soon after by an inflammatory reaction excited only by living tumor; the cell infiltration disappearing as the tumor became necrotic. In previously immunized non-susceptible mice the process of destruction was the same but began much earlier. Braunstein (6) concluded that the spleen possesses highly developed powers of resistance against tumor growth, while splenectomized animals are more liable than normal animals to inroads of malignant growth. Rohdenburg and Johnston (7) made parallel observations upon thymus, pancreas, spleen, hypophysis, and testis, showing that after extirpation of the thyroid, thymus, or testes, the animal had a lessened resistance to carcinoma.

Baeslack (8) studied the blood of inoculated animals and reported a decrease in the numbers of circulating lymphocytes in mice with growing tumors. Murphy (9), working with tissue cultures, noted that resistance to heteroplastic tissue grafts apparently depends upon the activity of the lymphocyte and obtained similar results with experimental cancer in mice. Murphy and Morton (10) in a series of experiments with transplanted and spontaneous tumors in mice noted that slight exposure to Roentgen rays, inducing a lymphocytosis, brought about a more highly resistant state to implanted tumor; whereas larger doses of the x-ray, inducing a leucopenia, caused a decreased resistance to inoculation with tumor, and that animals naturally immune were rendered susceptible by this method. They concluded that destruction of the lymphocytes by the x-ray causes a loss of the natural or induced resistance to the growth of inoculated cancer.

The apparently conclusive results of the investigators referred to above have not been without contradiction. Wade (11) noted that with the establishment and growth of an inoculable tumor, there is a steady increase in the percentage of lymphocytes in the circulating blood. Rous and Murphy (12), experimenting with sarcoma of the fowl, decided that no fundamental importance can be attributed to the protective function of lymphocytes in mammals and birds alike although they did state that the "lymphocyte has an association with the process of resistance in the fowl more marked than that seen in mammals."

Serafini (13) observed that ligation of the splenic vessels in rats and the injection of splenic tissue does not promote the growth of implanted tumor. Jones and Rous (14) noted that mice inoculated eight to ten days after splenectomy have the same resistance as normal animals; and that mice inoculated three and a half weeks after splenectomy live longer than normal animals. Bullock (15) and Stevenson (16) could not find that the presence of spleen inhibited the growth of tumor implanted into the chick embryo. Sittenfield (17), having utilized various means of inducing a hyperlymphocytosis, stated that "neither increase nor reduction of the lymphoid elements in the blood had any influence upon either resistance or susceptibility to tumor growth." Stevenson (18), again, found in 1917 that tumors show no inhibition of growth when grown on the allantois of the chick embryo for ten days in the presence of chicken spleen. Bullock and Rohdenburg (19) (20) also reported that removal of the spleen does not favor the growth of heteroplastic tumor grafts. More recently Prime (21) found that, following lymphocytic reduction by exposure to the *x*-ray, no appreciable decrease in immunity to tumor inoculation occurs.

As a result of these conflicting and widely divergent results we find that the rôle of the lymphocyte in immunity to cancer is still undetermined. The varying conclusions arrived at are due to the methods employed and to the instability of the leucocyte count in the lower mammals, for blood-cell counts in mice may be extremely variable. In attempting to devise a new means of approaching the problem, it occurred to the author that intraperitoneal inoculation of a tumor, preceded and followed by study of the cellular elements of the peritoneum, might yield information regarding the part played by the lymphocyte in cancer immunity.

The normal peritoneum of the white mouse contains numerous cellular elements, among which are found in predominating numbers the large mononuclear phagocytic cells designated as macrophages and small mononuclear cells having the morphology and staining reactions of lymphocytes. In addition there are large cells whose cytoplasm is filled with coarse basophile granules, resembling the mast cells of the blood, a few polynuclear leucocytes, and erythrocytes rarely. The macrophages, as nearly as can be determined by direct counts, are found to vary from 10,000 to 20,000 per cubic millimeter, whereas the lymphocytes are far more numerous, usually about 115,000 per cubic millimeter. In a few mice the macrophages predominate, but in the majority the lymphoid cells are more numerous. In differential counts made by obtaining peritoneal fluid with capillary glass pipettes, the average percentages found in a series of 24 mice were as follows:

	per cent
Macrophages	34.3
Large mononuclears	7.0
Lymphocytes	55.0
Polymorphonuclear neutrophiles	3.5
Basophiles	0.2
	100.0

The large mononuclears in the above classification are much larger than the lymphoid cells, but do not possess the abundant foamy cytoplasm of the macrophage. The designation of the small mononuclear cells as lymphocytes may be questioned. Evans (22) apparently refers to certain small mononuclears in the peritoneum as young macrophages, asserting that they will take up granules of vital stains, a characteristic lacking in true lymphocytes. This may apply to the cells referred to above as large mononuclears. Morphologically we find the small cells to be identical with the lymphoid cells of the blood. We do not find that they possess phagocytic properties for bacteria, inert particles, or vital stains; and finally, they decrease markedly under *x*-ray exposures.

It is very evident, then, that the peritoneum of the mouse contains large numbers of cells having all the characters of lymphocytes, and it is the purpose of the present investigation to show their influence on intraperitoneal inoculations of a mouse tumor obtained from the Crocker Research Fund, where it is designated as no. 180, and is regarded as a sarcoma. The tumor grows readily, and in our hands has not failed in more than 5 per cent of subcutaneous and 2 per cent of intraperitoneal inoculations. Inoculations were made by means of a small trocar needle.

The white mice selected were young adult animals; an equal number of each sex. Smears from the peritoneum were made before inoculation and repeated after inoculation till the "taps" became bloody and unfit for differential counting. Considerable variation in the percentage of lymphocytes occurred in the inoculated mice, and also some variation in the same mouse at different times. The lowest differential count recorded showed 25 per cent lymphocytes, the highest 80 per cent of lymphocytes, and the average in uninoculated mice 56 per cent lymphocytes. After inoculation, when the tumors were well developed and readily palpable through the skin, there was found an average of 54 per cent of lymphocytes. Twenty-two mice were used in this experiment. About half the animals showed a rise and the other half a fall in the percentage of lymphocytes following tumor inoculation. Table 1 illustrates the results obtained before and after inoculation. Following the inoculation, numerous polymorphonuclear leucocytes appeared in the peritoneum but not in sufficiently large numbers to influence visibly the percentages of the other cellular elements.

TABLE 1

MOUSE	PERCENTAGE OF LYMPHOCYTES		TUMOR
	Before inoculation	After inoculation	
18	60	75	Large
19	50	74	Large
20	30	56	Large
21	48	45	Large
24	80	75	Large
27	66	75	Large
30	60	64	Large
32	64	45	Large
33	65	85	Large
34	44	56	None
35	61	32	Large
36	54	40	Large
37	50	29	Large
47	56	53	Large
67	48	50	Large
68	66	70	Large
69	44	58	Large
70	49	31	Large
71	60	50	Large
74	80	70	Large
76	46	29	Large
77	40	42	Large

Of 22 mice, 11 show an increase in lymphocytes, and 11 a decrease.

Thus, as indicated in the table, of 22 mice inoculated with tumor all with one exception presented in a short time (ten to twenty days) rapidly growing tumor nodules. In making the punctures with capillary glass tubing blood was frequently obtained and when abundant the slides were discarded. The counts recorded above were made on relatively blood-free preparations. Since many mice showed an increase and others a decrease in the numbers of lymphocytes, and since the tumors were large and growing rapidly, it is apparent that no marked adverse influence can be attributed to the presence of the lymphocytes. The most important point brought out by the present investigation is the fact that small fragments of tumor tissue placed in a fluid rich in lymphoid cells showed no inhibition of growth.

CONCLUSIONS

1. The normal peritoneum of the white mouse is rich in cellular elements—115,000 to the cubic millimeter—of which about 55 per cent are lymphocytes.
2. Mouse tumor implanted into the peritoneum of these mice grows rapidly.
3. No marked change in the cellular content of the peritoneum of such mice results from the inoculation of mouse tumor.
4. No direct antagonistic action is found to exist between the lymphocyte and tumor implants in mice.

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PLATE 1

FIGS. 1 AND 2. Examples of rapidly growing peritoneal tumors following tumor inoculation into mice with numerous lymphocytes in the peritoneum.

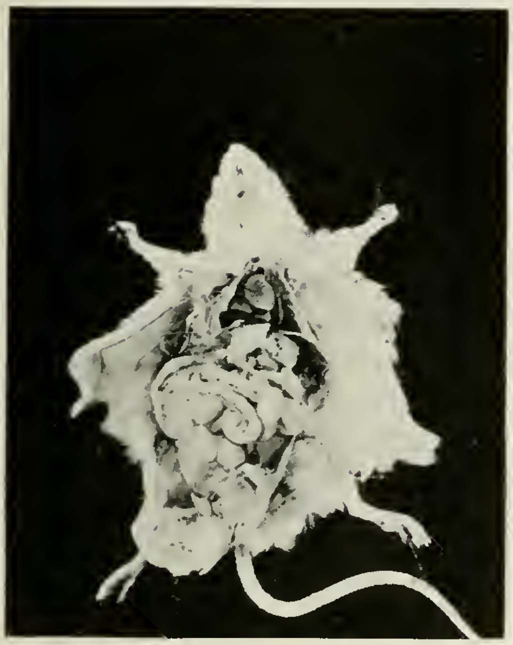

Fig. 1

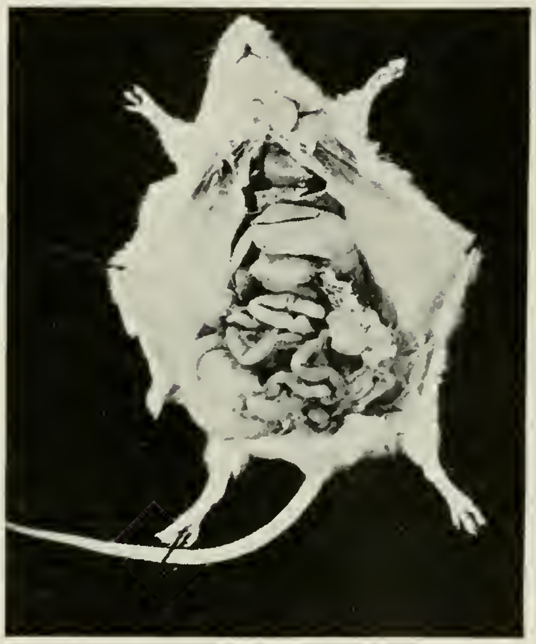

Fig. 2

PLATE 2

Figs. 3 and 4. Peritoneal smears from normal mice. Obj. 16 mm. Oc. 5. Note the large numbers of lymphocytes. The larger pale-staining cells are macrophages. A few mast cells are present.

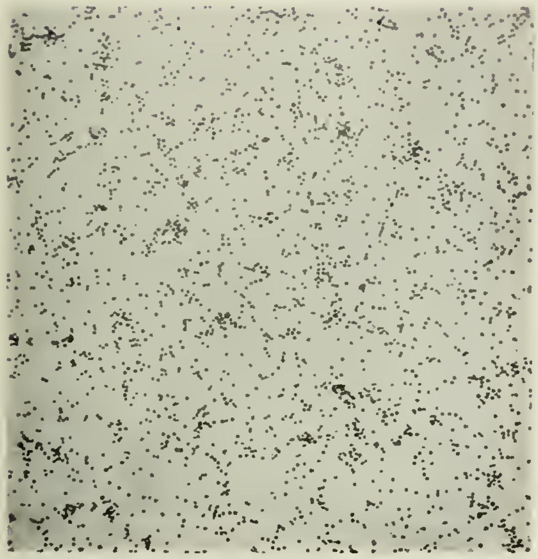

Fig. 3

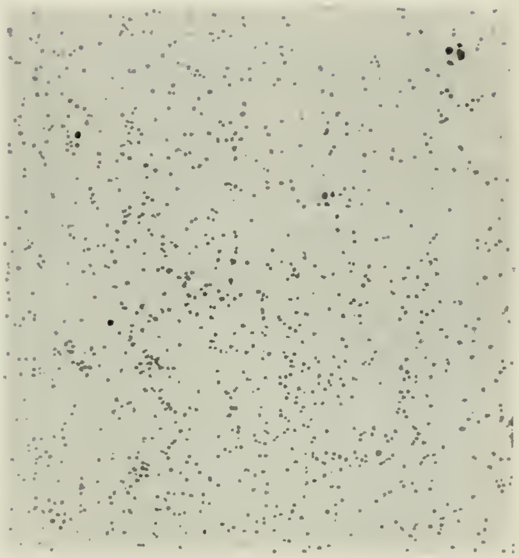

Fig. 4

PLATE 3

Fig. 5. Peritoneal smear showing large numbers of macrophages. Tumor growth rapid. Obj. 4 mm. Oc. 5.

Fig. 6. Peritoneal smear from mouse which showed 75 per cent of lymphocytes in presence of well-developed peritoneal tumor. Obj. 4 mm. Oc. 5.

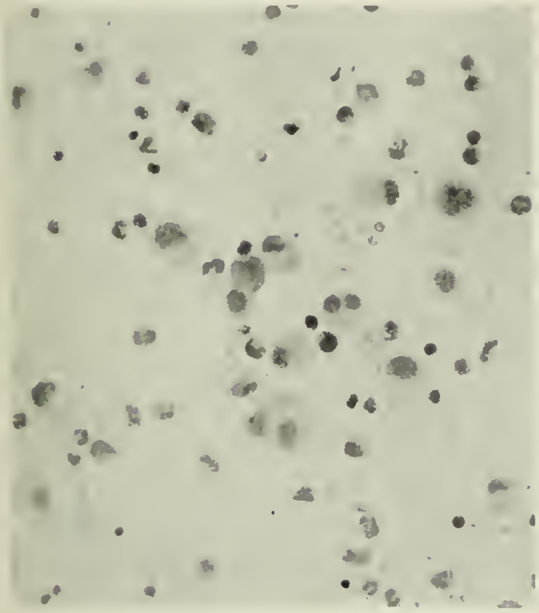

Fig. 5

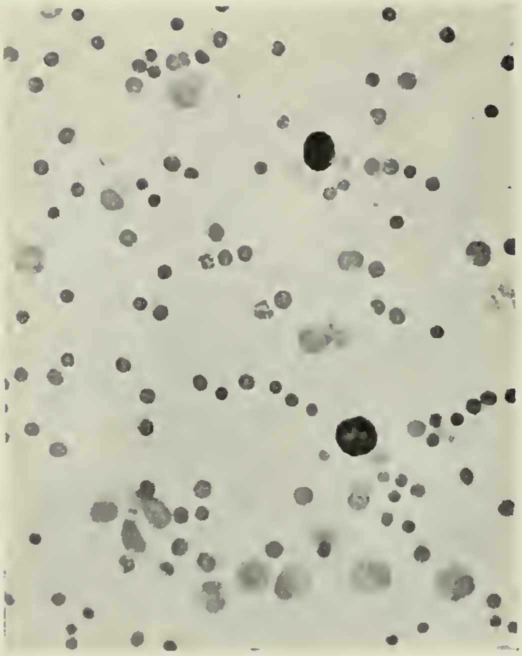

Fig. 6

PLATE 4

Fig. 7. Peritoneal smear showing 75 per cent lymphocytes. Tumor large and rapidly growing. Obj. 4 mm. Oc. 5.

Fig. 8. Peritoneal smear showing 70 per cent lymphocytes. Tumor large. Obj. 4 mm. Oc. 5.

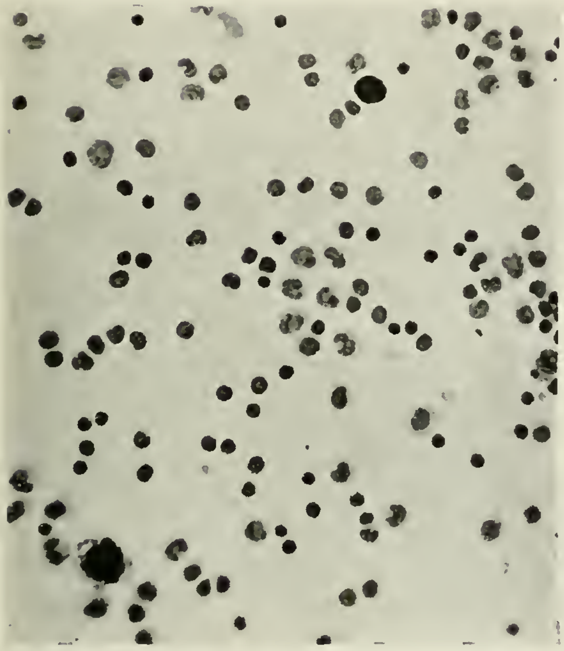

Fig. 7

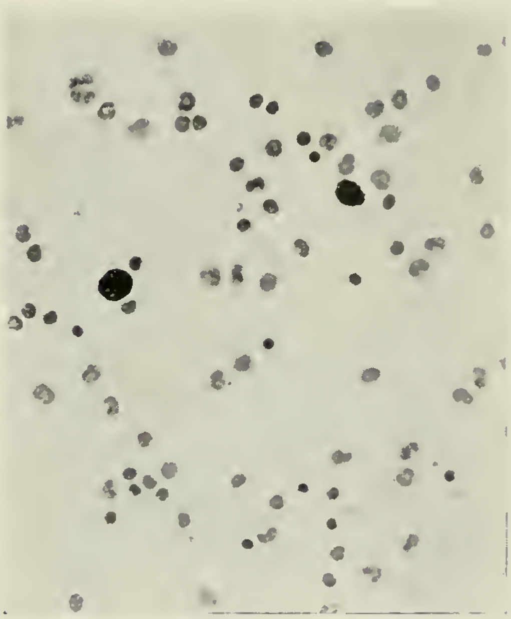

Fig. 8

PRIMARY SPONTANEOUS SQUAMOUS CELL CARCINOMAS IN MICE

STUDIES ON THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE

Fifteenth Communication

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Throughout most of the animal kingdom primary carcinoma of the skin is met with but rarely, in contrast with its great frequency in man, and also with the relative frequency of other cutaneous and subcutaneous tumors in animals. The existing literature on spontaneous tumors in animals indicates that no species except dogs, horses, and fowls, show skin carcinoma except as a rare condition, and this is of particular significance in view of the fact that skin carcinomas are more certain to be recognized than those in any other location and hence their relative infrequency is even more marked than any statistical evidence would indicate. Presumably the unprotected condition of the human skin accounts for its susceptibility to cancer, as the hairy scalp rarely shows primary carcinoma, and the pigmented skin of the negro also seems insusceptible.

The classical compilation of Sticker (1), which contains records of many tumors not fully differentiated according to modern criteria, gives the following relative frequency in this material of skin tumors in different species:

	SPECIES				
	Horse	Cattle	Dog	Cat	Swine
Total carcinomas	332	78	766	21	12
Skin	22	2	166	6	2
Eye	14	2	8		
Lip	4	1			
Anus	8		89	1	
Penis	52	2	16	1	
Vulva	11	1			

The extensive compilation by Teutschlaender (2) of the distribution of tumors in animals shows that cases of squamous cell carcinoma have been described in but few species, according to his records, namely: cat, dog, mouse, rat, elephant, beef, sheep, pig, horse, mule, cockatoo, toucan, chicken, carp, and gold fish. However, in another table he mentions also a case in a stag.

Apparently birds develop cutaneous carcinoma somewhat more often than most other animals, but even here there are but few recorded. In their summary of the literature on tumors in birds, Joest and Ernesti (3) found but 4 of 37 tumors described in birds to be skin carcinomas, those being two found on the legs of chickens by Wernicke; one on the wing of a "papapeis"—reported by Guerrini, and one on the wing of a toucan reported by Herbert Fox (4), this last case exhibiting also a metastasis in the lung. In none of their own 50 cases of tumors in birds was a skin carcinoma described. This compilation, as well as all others that we have observed, omits the striking case of squamous carcinoma of the skin of the foot of a hen described by Boynton (5), in which transplantations were made without success. More recently a similar tumor arising on the thumb claw of a lark has been reported by Urra (6). Two cases of squamous cell carcinoma of the mouth in fowls have been described by Pick and Koch. The experience of Teutschlaender (7) is unique, for he reports that of 54 carcinomas in fowls observed in the cancer laboratory at Heidelberg, 28 were skin epitheliomas, of which 18 were on the foot, attributed to the irritation of parasitic infection of the epidermis (*Cnemidocoptesmilben*).

Apparently fish are subject to skin tumors of various structure, including carcinoma. Thus, Fiebiger (8) reports cases of epithelioma occurring on the lip of each of two "Schlei" (*Tinca vulgaris* Cuvier) coming from the same pond, a markedly malignant skin epithelioma in a carp, and mentions papillomatous growths in several specimens of Kletterfish (*Anabus scandens*). Plehn has also described numerous skin epitheliomas in cyprinoid fishes.

Bashford has reported a squamous cell carcinoma in a small fish, *Gasterosteur spinachia*, two cases of carcinoma of the skin in frogs, and a carcinoma of the skin glands in a Triton.

Among mammals the dog alone seems to be commonly subjected to skin carcinoma, if we except the carcinoma of the penis and prepuce occurring not infrequently in horses and oxen. It would seem to be not uncommon in cats according to the experience of Roffo (9), who found 7 tumors in 307 cats examined, of which 4 were skin carcinomas on the head and face, with lymph gland metastases, but none of 11 cases of tumors in cats collected by Bashford (10) was of this type, and but 2 of 48 tumors in dogs, although there were several squamous cell growths in the mouth and pharynx. Leo Loeb (11) reported in 1903 that from one to three carcinomas of the inner canthus of the eye were observed every week in the Chicago stock yards, but in one year no other external carcinomas were observed except two of the vulva.

Rodents seem to have skin carcinoma but rarely. The large series of wild rats autopsied in plague work and examined for tumors (McCoy, Woolley and Wherry, Beatti) have revealed no such growths except for an epithelioma of the vulva reported by Woolley and Wherry (12). It will be recalled that the early transplantation experiments of Hanau (13) were with a squamous cell carcinoma from the vulva of a white rat, and he states that there had been two previous cases of similar growths in rats in their laboratory. Roffo (14) has also observed a squamous cell carcinoma which arose in the vulva of an old white rat, which was transplanted through six generations without change in structure. In view of the great frequency of parasitic skin infection in rats, "rat scabies," commonly with extreme papillomatous proliferation of the epithelium (15), it is strange that epithelioma has not been more frequently observed. Of 123 cases of spontaneous rat tumors reported in the literature and collected by Bullock and Rohdenburg (16), there were but three epitheliomas, one of the tongue and two of the vulva. They reported on 48 rat tumors observed in the Crocker Laboratory, one of which was an epithelioma of the head, which was not described further. Morris (17) has reported a basal cell carcinoma which arose in the skin of a male rat, five months old, and which died out after two generations of transplants. He was unable to find other recorded instances of basal cell carcinomas in animals.

Several instances of squamous cell carcinoma have been described in mice, but they form an insignificant proportion of the many spontaneous tumors that have been observed in this species. Nevertheless they were looked upon as of great significance in the early days of experimental cancer research, since they established the fact that mice had other forms of tumor than the predominating mammary gland carcinoma. Haaland (18) reported a case of squamous cell carcinoma arising in the mouth of a mouse in Borrel's laboratory, involving the lower jaw, showing a typical squamous cell structure. Six mice were inoculated from this, but as in six months they showed no growths they were put back into the cage with the others. Ten months after the inoculations "deux cas identiques de cette même tumeur apparurent en même temps dans cette cage," but it was not known whether these were inoculated mice or not and Haaland does not make clear whether these later tumors were in the mouth, although this is apparently what he means. Later he observed a third case of mouth cancer in a mouse recently inoculated with a Jensen tumor. No growths developed in mice inoculated from this mouth tumor.

Murray (19) in 1908 described the following cases of superficial squamous cell carcinoma in mice: (1) A prickle cell growth with apparently little keratinization, arising in the skin of the neck, with metastasis into an adjacent lymph gland. (2) A carcinoma presenting both alveolar and squamous cell areas, with keratinizing metastases in the lung. Presumably this tumor arose in the mammary gland. (3) Prickle cell growth without keratinization, interpreted as primary in the nipple; this mouse also had had a hemorrhagic adenocarcinoma of the mammary gland removed by operation. In discussing the mammary gland tumors he also describes the occurrence of areas of keratinization within tubular carcinomas, and regards them as indications of the close association of the mammary apparatus with the skin from which it develops, and points out that the ampulla which receives the terminal portions of the mammary ducts is also lined by stratified squamous epithelium. "Therefore, should the cells of the new growth have taken their origin near the nipple, variations in either direction are only to be expected."

In his later report from the London laboratories, Haaland (20) states that of 353 spontaneous tumors observed (not including those in Murray's report), 22 were squamous-celled carcinomas with marked keratinization, of which 14 arose in the mammary region and 8 outside it. Of these 8, 3 were mouth tumors similar to those observed in Borrel's laboratory, apparently arising in the mucous membrane of the inside of the cheek, there being in one metastases in the lymph nodes. One seemed to have arisen on the surface of the head, and it penetrated the skull; grafts from this were successful. (The illustrations of these tumors show the gross relations of such tumors so well that we are spared the necessity of reproducing further illustrations.) Three tumors were described as superficial verrucous growths, on the vulva, anus, and left flank. One was a typical squamous cell carcinoma of the skin of the chest, developing as a superficial ulcerating growth, "undoubtedly from the skin itself, or from the mammilla." A case of mixed squamous and sebaceous carcinoma of the vulva is also mentioned.

Several of the keratinizing mammary tumors differ from typical squamous cell carcinomas, and their cutaneous origin is doubtful. Four are described as resembling "molluscoid" tumors, characterized by long radiating cylinders of cells with a central keratinized zone, found in the mammary region and not giving successful transplants. Four tumors are described as "typical squamous-celled alveolar carcinomata in the mammary region." Six others are called "adenocancroids," these being unquestionably mammary gland adenocarcinomas in which areas of keratinization occur. Attention is called to the occurrence of squamous-celled cysts in the normal mamma, apparently arising from mammary ducts in which the epithelium has become metaplastic, and which perhaps explains the occurrence of keratinizing carcinoma in the mammary gland. As squamous-celled growths have also been found rarely in the human breast, as well as in cats and dogs—Teutschlaender (21)—the disease in mice is not without analogy, although apparently relatively frequent among the mammary gland tumors of this species. Presumably in some stocks of mice keratinization is even more common in mammary gland carcinoma than the above reports indicate, for Woglom (30) found this change in 228 of 1000 spontaneous mammary gland carcinomas examined in the Crocker Laboratory.

Pick and Poll (22) reported as a "sweat gland carcinoma" a tubular growth arising near the scapula of a mouse, but the description published does not permit the exclusion of a mammary gland origin.

Erdheim (23) described a pedunculated tumor, which gave no evidence of malignancy, but exhibited a histological structure resembling squamous cell carcinoma. It arose from the vulva of a mouse, and after the outer part of the growth had been removed the base healed spontaneously. He was unable to classify this tumor, beyond indicating that it was a stratified epithelial neoplasm.

Tsutsui (24) has produced carcinoma on the skin of the back of mice by painting with tar, after the procedure used by Yamagiwa with the ears of rabbits, observing 16 carcinomas and 1 sarcoma in 17 mice surviving over 100 days. In two cases lung metastases were observed.

We find no other reports of squamous cell carcinoma arising in the skin or mouth of mice, which indicates their relative infrequency, since such tumors are most obvious when they do occur. Thus, Tyzzer (25) in his report of 83 spontaneous tumors in mice includes no tumors of this type. In the 41 primary mouse tumors described by Jobling (26) there were no skin epitheliomas, although there was one of the molluscoid keratinizing tumors such as Murray, Haaland, and Tyzzer have described.

Squamous cell carcinomas outside the mammary gland and skin of mice are rare. Tyzzer reported that among his lung tumors "in several cases" the growth was of an epidermoid character, and Haaland described one such tumor. In our previous paper on primary carcinoma of the stomach in mice (27), we collected four cases of squamous cell gastric carcinoma reported in the literature and added three more in our own material, as well as one case of squamous carcinoma in the external surface of a chronically prolapsed rectum. Fiebiger (31) has also reported the occurrence of a few instances of squamous cell carcinoma produced experimentally in the stomach of mice by feeding cockroaches injected with Spiroptera. We have found no reports of squamous cell carcinomas arising in the esophagus or urinary tract, or in the cervix uteri, or in any other structure where they might be found in mice, except such as have been mentioned above.

OBSERVATIONS ON SKIN TUMORS AND SQUAMOUS CELL CARCINOMAS IN THE SLYE STOCK OF MICE

In 28,000 consecutive autopsies performed on mice of this stock, which had been permitted to live as long as possible without any experimental manipulations whatever, we have observed the following instances of primary neoplasms of squamous or stratified epithelial structure.

Primary squamous cell carcinoma of the skin and mouth	70
Primary basal cell carcinoma of the skin	15
Primary keratinizing carcinoma of the mammary gland	56
Primary squamous cell carcinoma of the stomach	4
Primary keratinizing carcinoma of the lung	1
Primary squamous cell carcinoma of the rectum	2
Primary squamous cell carcinoma of the vagina	1
Primary stratified carcinoma of the meibomian gland	2
Primary sebaceous gland adenocarcinoma	1
	152

As we have not yet made a complete analysis of all the tumors observed in these 28,000 autopsies the proportion of stratified cell tumors to total tumors cannot be stated. Roughly there are probably about 4000 primary spontaneous tumors of all sorts, so that these squamous cell tumors constitute not far from 4 per cent of the total.

CARCINOMA OF THE SKIN

In our material are 85 malignant epithelial growths arising in the skin, mouth, and lips, excluding those squamous cell growths that seem to be derived from mammary gland tissue and stratified cell growths from other glands. Of these, 70 are of squamous cell type and 15 of basal cell character. Since many of the tumors which arise about the lips and mouth are, when observed, so extensive that their exact point of origin cannot be determined, we have grouped them together with the skin tumors. Roughly classifying the site of the 70 squamous cell growths, 13 were in the skin of the trunk, one on a front limb, 2 on the vulva, 15 about the lower jaw, 18 on some other portion of the face, and 23 about the ears or neck; that is, all but 16 of the 70 were on the head and neck. Presumably this is to be explained by the much greater amount of traumatism suffered by the skin in this part of the body, which is especially marked in cage mice which are always rubbing their muzzles against the rough wire meshes of the cages. The tumors of the jaw and mouth in several instances seemed to have resulted from the irritation produced by broken or protruding teeth. Nearly all of the carcinomas arising on the trunk were definitely located at the site of a healed wound, and in not a few of those of the head the same origin was observed; it is probable that less obvious wounds are responsible for many if not all of the others. A chronic dermatitis often preceded the carcinomas of the skin of the face, presumably incited by traumatism. The not uncommon fungus infections of the skin seem to be too rapid in their course to lead to carcinoma formation.

Clinically these growths are similar to corresponding forms of carcinoma in man (fig. 1). The age at which they occur is, on an average, later than with any other mouse tumors, as they rarely appear before late middle life, and usually only in old age. They arise under crusted ulcers, or develop in areas of hyperplasia from chronic irritation, spread slowly as crusting, ulcerated growths, destroying at times the eyes or other features, usually remain superficial, and most often cause death by chronic infection of the ulcerated surface, which may also lead to acute abscess formation. Occasionally death results from starvation when the cancer involves the mouth or jaw, from hemorrhage, invasion of the skull, or other coincident diseases.

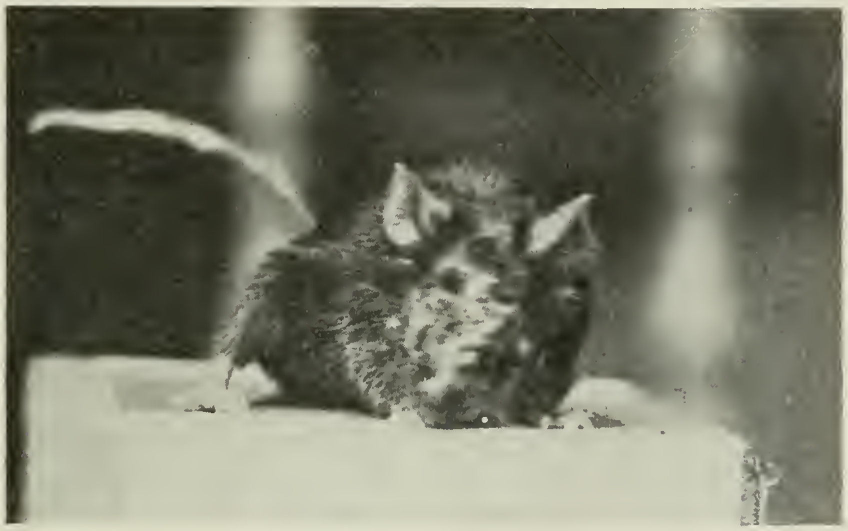

FIG. 1. HEAD AND FACE OF MOUSE WITH EXTENSIVE BASAL CELL CARCINOMA OF THE SKIN, OF AT LEAST FIVE MONTHS DURATION. No. 7874

The microscopic diagnosis of the skin neoplasms is often difficult, especially with the basal cell growths, since we find all stages of epithelial overgrowth from simple hyperplasia to metastasizing carcinomas. In this series no growth is included as a carcinoma unless it exhibited both the gross and clinical features of malignancy together with distinct microscopic evidence of infiltrative character. The extensive destruction of these growths by infective processes often obscures the microscopic findings. In several instances a growth that presented

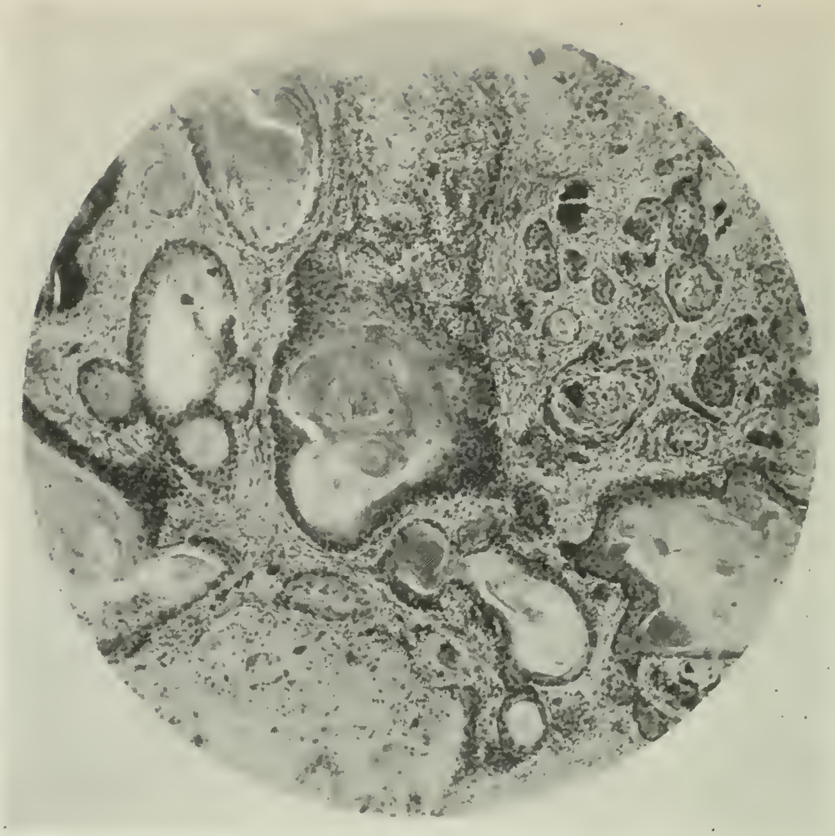

Fig. 2. Typical Squamous Cell Carcinoma of the Jaw, with Formation of Abundant Epithelial Pearls. No. 23331. × 60

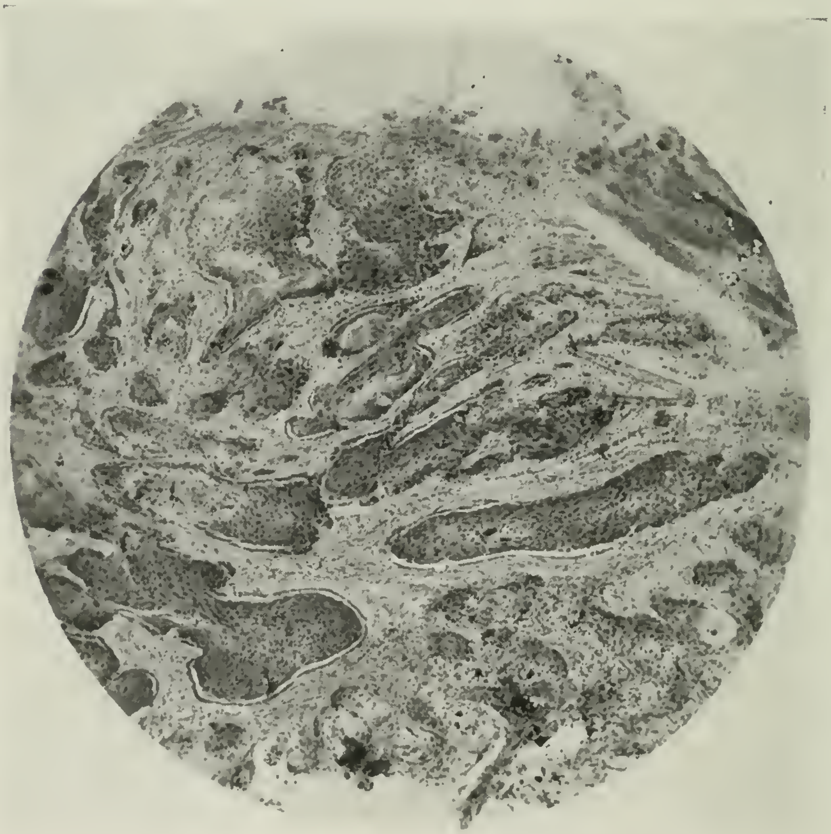

Fig. 3. Squamous Cell Carcinoma of the Jaw Infiltrating Adjacent Tissues Extensively. Type with Little Hornification. No. 26238. × 60

all the clinical features of carcinoma has been found at autopsy so extensively necrotized and suppurating that only examination of numerous sections from different parts of the growth has established the diagnosis of carcinoma. It is quite possible that we have lost a few genuine carcinomas through such destructive processes, to say nothing of cases in which the cannibalistic mate has selected the neoplasm for the first course.

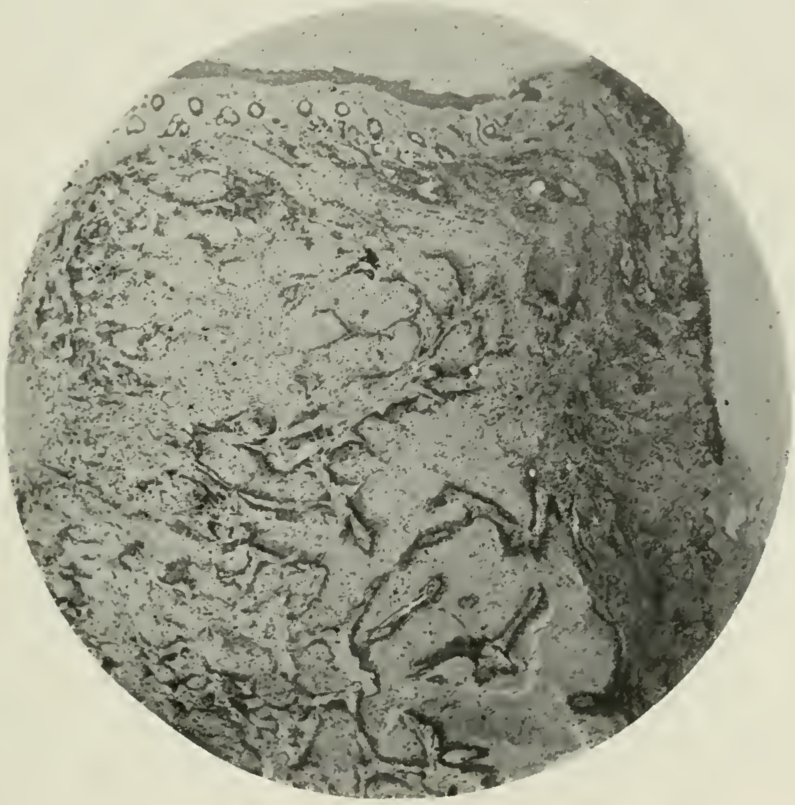

Fig. 4. Squamous Cell Carcinoma of Skin of Chest Arising in the of an Old Wound, the Scar Tissue Being Abundant in this Field, and the Relation of the Cancer Growth to the Overlying Skin Being Shown. No. 8212. × 45

The microscopic features of the squamous cell carcinoma the skin and mouth of mice differ not at all from those that occur in man. Usually hornification is marked (fig. 2), but, as in man, growths from a similar origin may show little or no tendency to form keratin (fig. 3). Usually the amount of connective tissue formation is not large, except in some cases where the cancer has developed in scar tissue from old wounds (fig. 4). Little help in diagnosis is afforded by mitotic figures, since these are scanty in even the most typically malignant growths, and commonly cannot be found at all. Presumably they would be much more numerous if the tumors were removed from the living animal.

The infiltrative character of growth is not usually so extensive as we are accustomed to see it in man, for the mice generally succumb to infection from the ulcerated growth while it is still small. Infiltration is usually seen best in the tumors that arise about the jaws, for here the bone is often invaded. Bone infiltration was observed in 7 of the 15 tumors that arose about the jaw. We have had one striking case (23766) in which a carcinoma of the skin of the head, beginning at the base of the right ear, infiltrated the skull and cervical vertebral canal, infiltrating the meninges about the cerebellum and compressing the spinal cord in the cervical region (fig. 5). This case resembles

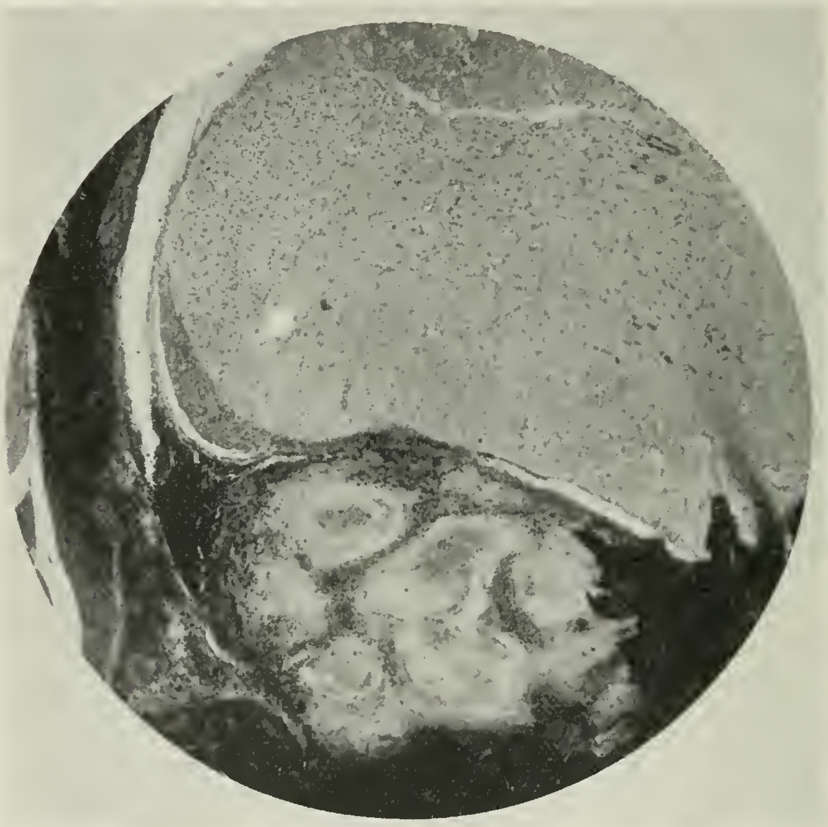

Fig. 5. Squamous Cell Carcinoma of the Head and Face Invading the Spinal Column in the Cervical Region, Compressing the Spinal Cord and Infiltrating the Meninges

This tumor also invaded the skull. No. 23766. × 60 the one described and pictured by Haaland, in which the skull was invaded. In two instances we have seen infiltration of the salivary glands, and in three cases an adjacent lymph node was involved apparently by direct extension.

The absence of lymphatic metastasis is a striking feature of these tumors when compared with corresponding growths in man, which is true of all forms of carcinoma in mice. In only two cases did we find a secondary growth by metastasis into a lymph node from a skin carcinoma (7950, 15232), and it was a very common observation that lymph nodes immediately adjacent to or in contact with these squamous cell growths, were not involved. Possibly serial sections of all our mice would have revealed other instances of metastasis, but the value of the information did not seem commensurate with the labor involved. In only one case did we find visceral metastasis (12627). This mouse had a squamous cell carcinoma arising just dorsal to the rectum, and a typical secondary nodule about 1 mm. in diameter in the lung; it also had a tubular carcinoma of the mammary gland which had produced no metastasis. The infrequency of metastasis in this series of spontaneous squamous cell carcinoma is significant when compared with Yamagiwa's experimental tar tumors in rabbits, since the infrequency of metastasis in his material has been thought by some to speak against their being true malignant tumors. As a matter of fact, the metastasis incidence obtained by him, and by Tsutsui with experimental skin tumors in mice, is distinctly higher than that observed in these spontaneous growths.

The sex incidence is strongly in favor of the female (49 to 21) in this series. As most of the growths were on the head and neck we cannot account for this on the basis of erroneous inclusion of squamous carcinomas of the mammary gland with our skin tumors. It differs from our experience with other growths of non-reproductive tissues which have shown approximate equality as to sex incidence. Even more than with other tumors we have found that age is an important factor, these squamous cell carcinomas of the skin being predominatingly in old mice, and as many male mice die early from wounds received in fighting this may account for the relative preponderance of females in this series.

Beyond the relatively slight extent of infiltration and metastasis these carcinomas present no noteworthy differences from human skin carcinomas. Often the amount of keratin scales piled up on the surface is strikingly great, and in one case the carcinoma arose at the base of definite cutaneous horns (25785). There is often a noteworthy amount of calcification of the necrotic scales. Cyst formation is frequently observed, and benign cutaneous cysts have been observed in several mice.

The basal cell carcinomas all arose on the face, ears, and head (fig. 1), 9 of the 15 being in females. Because these growths are of relatively low malignancy, as in man, they present particular difficulty in diagnosis, and we have set aside as "precancerous" numerous instances of basal cell hyperplasia of marked degree which did not present unqualified proof of malignancy. Quite frequently enormous hyperplasia of the cells about the hair follicles produces tumors of considerable size, and in these may be found areas highly suggestive of malignancy, in that altogether atypical plugs and masses of basal cells are formed, as shown in figure 6. Such growths, which very probably would have shown unqualified malignancy had the mouse lived longer, have not been included among the basal cell carcinomas. In general, basal cell carcinoma in the mouse corresponds entirely to the corresponding growth in man (fig. 7). To quote MacCallum (28), "In spite of the complexity of the downward growing strands all reach to about the same level. Further, it is seen that they are very sharply outlined against the stroma and show little inclination to strew their cells into the irregular crevices of that tissue." We have found the various modifications of basal cell growths commonly described, such as the formation of tubules suggestive of undeveloped hair follicles, hornifying surface areas with typical basal cell growths beneath, and the so-called adenoid epithelioma which seems intermediate between basal and squamous cell growths. Among our keratinizing carcinomas of the mammary gland was one of cells that suggested a basal cell character. No metastases were

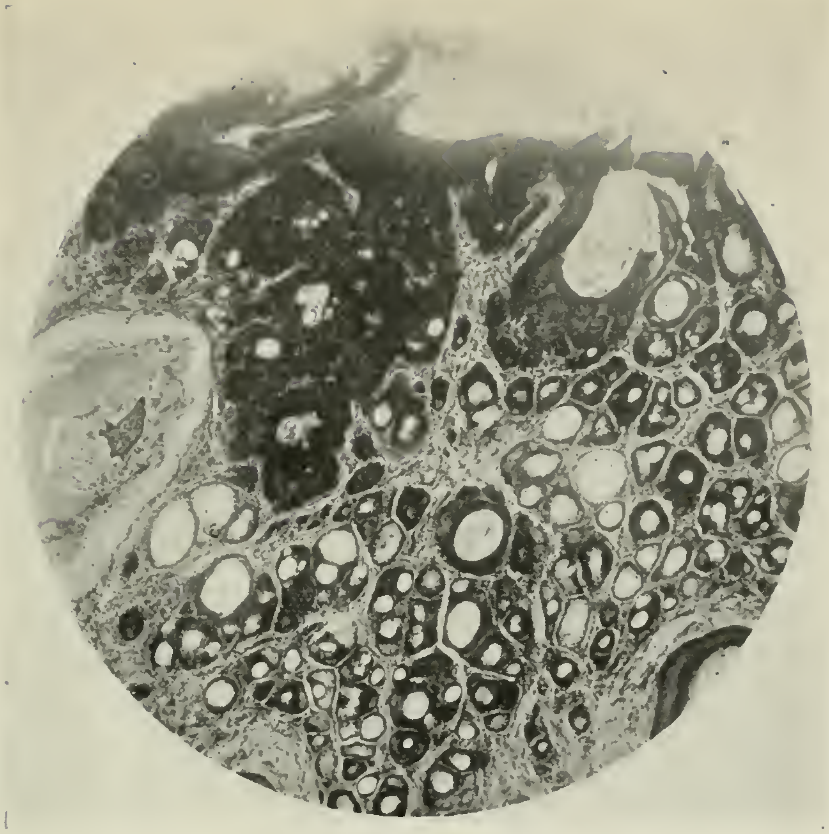

Fig. 6. Basal Cell Hyperplasia of an Irritated Muzzle, with Areas of More Atypical Growth

There have been numerous such growths, which are probably properly designated as precancerous, but may be actually malignant. No. 16563. × 60.

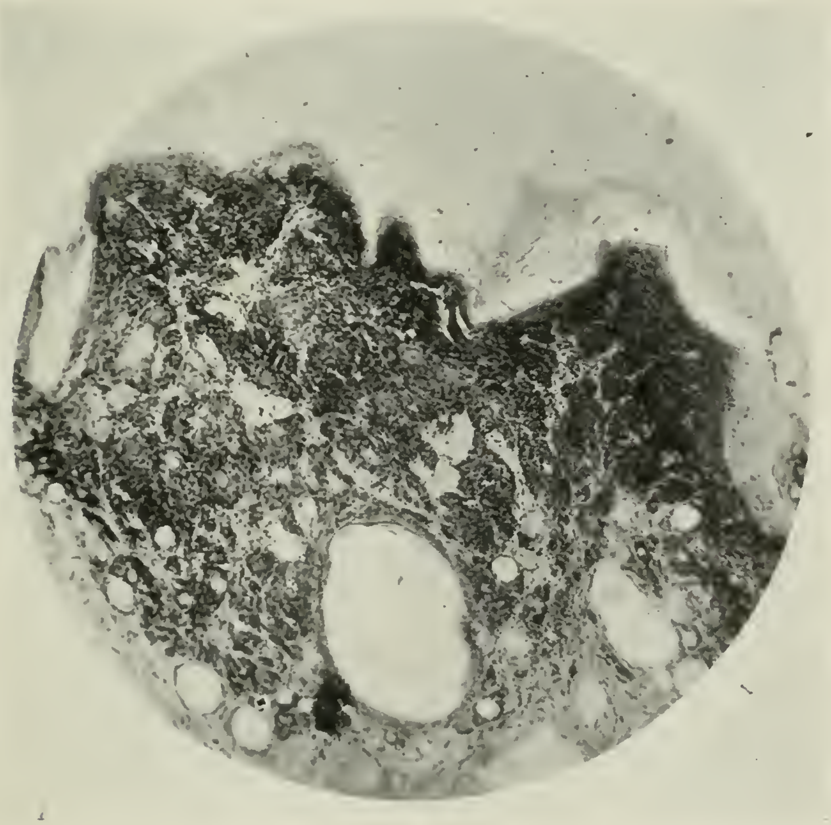

Fig. 7. Basal Cell Carcinoma Which was Highly Malignant, as It Entirely Replaced the Face, Destroying Both Eyes, and Forming Tumor Masses in the Orbits. No. 9932. × 90

observed among these cases, although in one, extensive infiltration destroyed both eyes and produced large tumor masses in the eye sockets. Several of these growths arose at the site of distinct scars from wounds.

Despite the fact that these skin tumor mice were all well advanced in age the co-existence of other tumors is lower than that seen in many other forms of mouse tumor. Perhaps one factor in this is that most of the mice with skin cancer had lived beyond the age at which other tumors occur most frequently. It also seems that the heredity of these mice is responsible to some extent, but a complete analysis of this factor has not yet been made. Of the 85 cases, in which 58 were females and 27 males, but 6 showed a carcinoma of the mammary gland, 7 exhibited lung adenoma (including one that also had a mammary gland carcinoma), one had an adenoma of the ovary and one seemed to have pseudoleukemia. Despite the number of old wounds in these mice no cases of sarcoma were observed, presumably because of either old age or ancestry, but one case of carcinoma arising in the mouth and infiltrating the jawbone, showed such a flattening of the deeper cells that for some time we were in doubt whether or not it was an instance of mixed sarcoma and carcinoma. This case (8560) is described and pictured in our article on sarcoma in mice (29).

SQUAMOUS CELL CARCINOMAS OF THE MAMMARY GLAND

The keratinizing carcinomas of the mammary gland form an interesting group, and properly lie outside the scope of this paper, except for the fact that with not a few of them it is extremely difficult to tell whether we are dealing with a primary mammary gland carcinoma or a skin carcinoma arising over the mammary gland. Three types of these tumors can be distinguished.

1. Carcinomas of the mammary gland which are essentially cylindrical cell carcinomas, forming tubules and alveoli, but some areas of which undergo a transformation into stratified epithelium with the formation of keratin, often in large amounts (fig. 8). These are the "adenocancroids" of Murray and

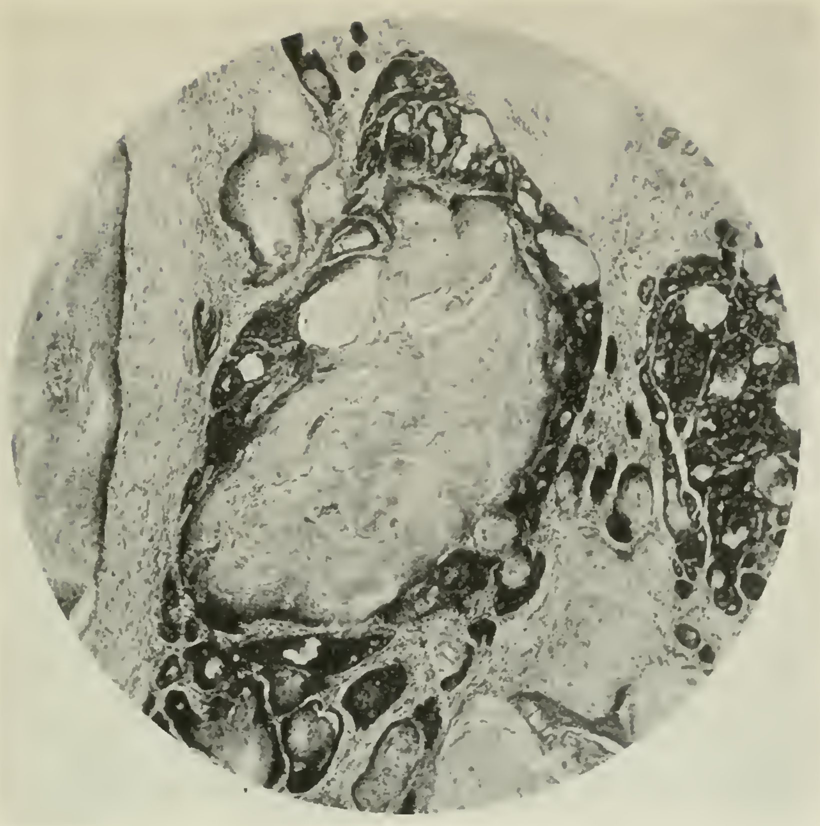

FIG. 8. TUBULAR ALVEOLAR CARCINOMA ("ADENOCANCROID") OF THE MAMMARY GLAND WITH AREAS OF KERATINIZATION

To the left is seen a band of densely keratinized tissue with no evidence of its glandular origin. No. 13336. × 60.

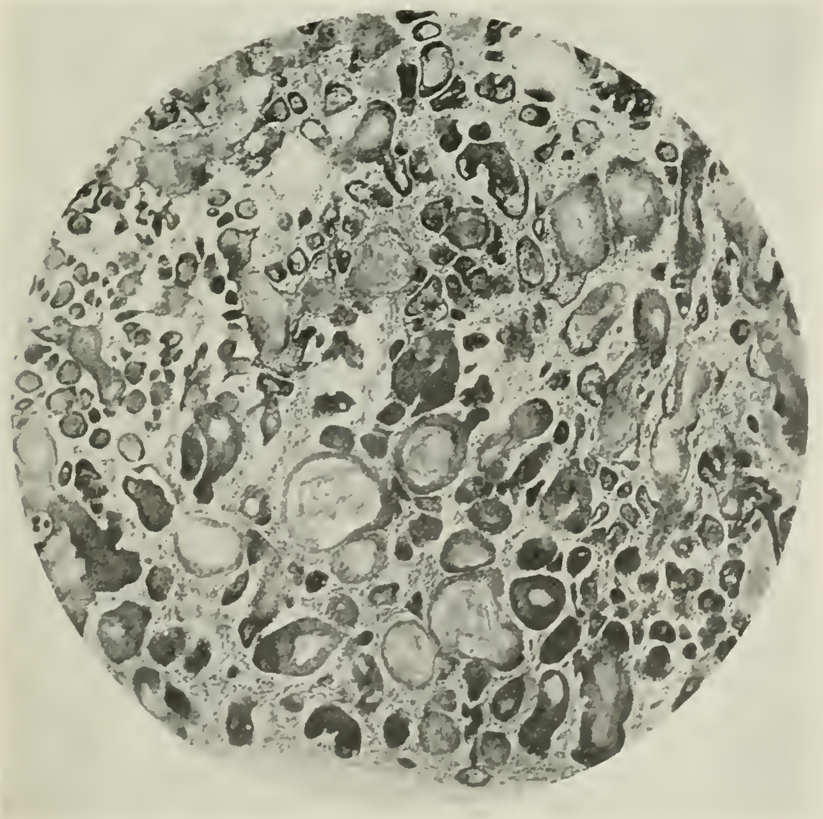

FIG. 9. SUPERFICIAL ADENOCANCROID, APPARENTLY ARISING IN THE DUCTS OF THE MAMMARY GLAND

Shows very little evidence of origin in glandular tissue. This mouse also had three other tubular carcinomas of the mammary gland without keratinization No. 15300 × 60.

Haaland. It is probably of some significance that the keratinization is usually most marked in the portions nearest the cutaneous surface, as if it began in the ducts. The amount of keratinization varies, sometimes appearing in only a few small spots in the tumor tissue, but often extending until little of the original columnar cell type of tissue remains.

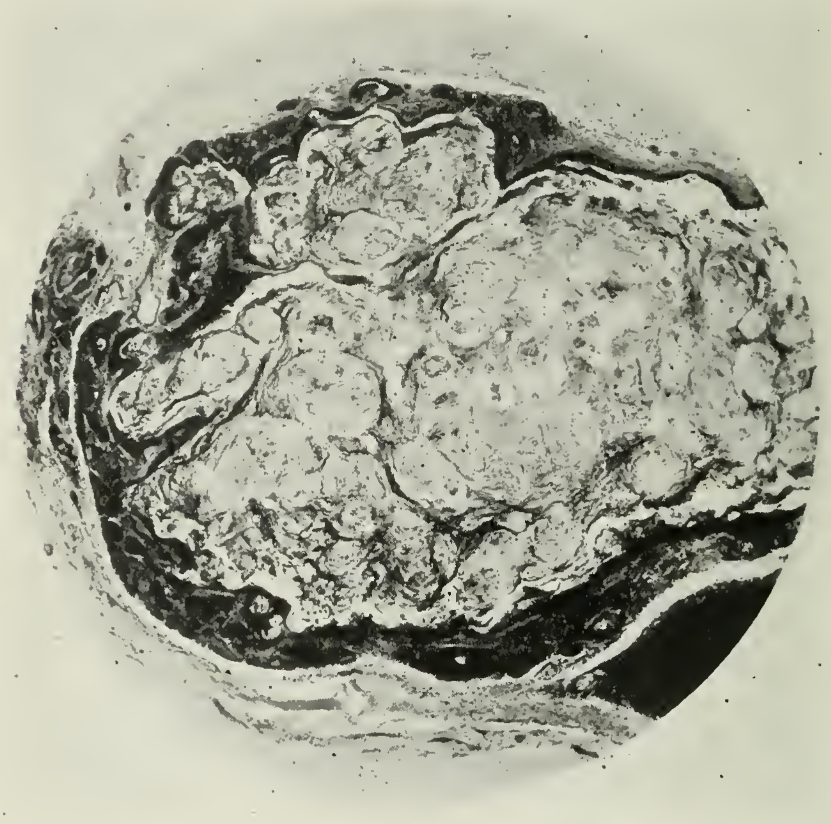

FIG. 10. KERATINIZING CYSTS IN THE MAMMARY GLAND
Such structures are not infrequently found, and may be the precursors of some of the malignant keratinizing carcinomas of the mammary gland. No. 8271. × 40.

2. Squamous-celled keratinizing carcinomas without any evidence whatever of cylindrical cell structure, but arising subcutaneously in the mammary regions (fig. 9). Many of these present no histological evidence that they are derived from the cells of the mammary gland, but they are observed to arise within the gland substance and sometimes are still entirely subcutaneous when the mouse dies. The occasional presence

within the mammary gland of what seem to be simple benign cysts of stratified epithelium with masses of desquamated hornified material (fig. 10) indicates the probable origin of such tumors. When they ulcerate on the surface it may be impossible to differentiate them from primary carcinoma of the skin or nipple, for histologically they differ little if at all from the usual squamous cell carcinoma of the skin. When observed early their origin in the gland beneath the skin is usually the chief ground for recognizing them as mammary gland tumors; nevertheless, the fact that after differentiating, on a histological basis solely, the skin carcinomas from the mammary gland carcinomas we found that nearly all those we had selected as of cutaneous origin had

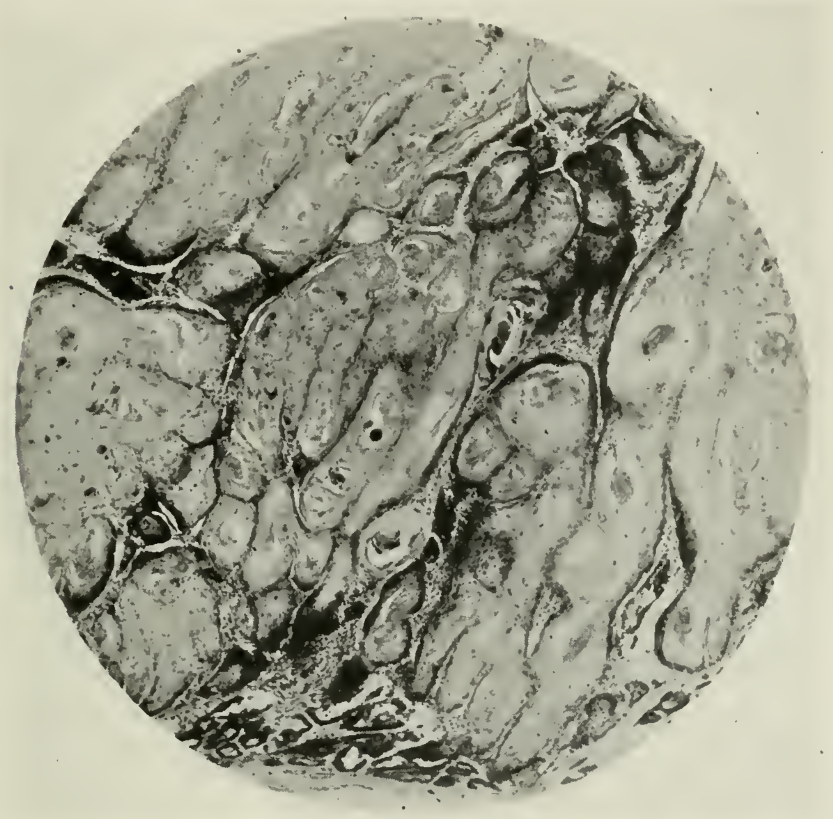

FIG. 11. MOLLUSCOID CARCINOMA OF THE MAMMARY GLAND

This growth, which arose in an inguinal mammary gland, invaded the abdominal wall and protruded into the pelvis, but produced no metastases. A non-keratinizing carcinoma of the mammary gland was also present which produced pulmonary metastases. No. 15622. × 60.

arisen at the site of old skin wounds, indicates that there are usually recognizable differences.

3. Molluscoid carcinomas of the mammary gland, to adopt Borrel's designation of these striking growths with long, radiating cylinders of stratified epithelium with a large central core of keratinized material (fig. 11). These seem to grow out from the tubules near the nipple, as if they arose from the stratified portion of those ducts. They produce ulceration of the surface about the nipple, which greatly enhances their resemblance to the skin carcinomas.

We have little to add to the statements of Borrel, Murray, Tyzzer, and Haaland concerning these squamous cell growths arising within or about the mammary gland, and the excellent illustrations accompanying Haaland's article make any further illustrations by us unnecessary. A few points may be worth recording. Forty-three of the 56 squamous-celled mammary gland tumors may be best designated as of the adenocancroid type, in that they show a greater or less proportion of cylindrical cell structure. Several of these were multiple, as is often the case with primary carcinoma of the mammary gland. In three there were two adenocancroids, one mouse had three, and one had five, four arising distinctly in mammary gland tissue while the fifth arose anterior to the urethra and seemed to have originated in the skin, and this showed no glandular elements. Fourteen mice had an adenocancroid and also one or more other independent mammary gland carcinomas that showed no keratinization. Of these, one (9365) exhibited also a typical squamous cell carcinoma metastasis in the lung; one mouse with three primary adenocancroids (5738) showed a lung metastasis while three mice that had each two mammary gland tumors, one of each type, had pulmonary metastasis of the cylindrical cell type. That is, from 43 cases of adenocancroid of the mammary gland, but one metastatic nodule of adenocancroid has been seen in the lungs, and none in the lymph nodes or elsewhere. Not a few of these mice had also co-existing tumors other than those of the mammary gland, namely, one with a squamous cell carcinoma of the muzzle (9962), one with a malignant ovarian tumor

(6801), two with uterine fibroids (14370 and 14811); one with a lung adenoma (12098); one with an adenoma of the liver and an adenoma of the lung (9544); one with an adenoma in the lung and a strange growth in a subcutaneous lymph node that might be interpreted as an endothelioma (15622) and one had an intrapelvic sarcoma with metastasis into the liver and also into an ovary which contained in addition a small adenoma (10006).

Two of the adenocancroids were examples of the not uncommon mixture of sarcoma and carcinoma in the mammary gland of the mouse (15412 and 6182).

Only four of our tumors were typical of the molluscoid cancers, although more or less of this character was shown by several of the growths diagnosed as adenocancroids. One of these four mice had also an adenoma of the ovary (496) and another had a benign lung adenoma (5581). None of these four keratinizing tumors produced metastases.

Nine squamous cell carcinomas that exhibited no glandular structure to identify them as mammary gland tumors, were believed to have this origin because they arose beneath the skin, and in most instances did not ulcerate through at any time. These growths, however, seem to be of a more malignant character than the adenocancroids; three of them produced squamous cell metastases. No. 10936, which is remarkable in being the only male with a subcutaneous stratified epithelial growth that seemed to arise in the mammary gland, exhibited squamous cell metastatic growths in the lung, in the mediastinum, and in the chest wall. Another (7950), with three subcutaneous, nonglandular squamous cell carcinomas, showed a metastatic nodule in one lymph node and direct infiltration of a second. No. 13671 with a subcutaneous squamous cell carcinoma and also a cylindrical cell mammary gland carcinoma, had three large metastatic nodules in the lungs all of squamous cell structure. As to multiplicity of tumors in this group there were three independent subcutaneous squamous cell tumors in each of two mice (2239 and 7905), two had a simple cylindrical cell carcinoma of the mammary gland in addition to the squamous cell growth (7526 and 13671), while one had adenomas of the liver and ovary

(14047) and one mouse had leukemia (8236). In this group we have included one squamous cell tumor (24858) that differs from the rest in having the non-keratinized tumor elements suggest strongly the appearance of a basal skin carcinoma, although the growth seemed to arise in the mammary gland.

SQUAMOUS CELL CARCINOMA OF STOMACH AND RECTUM

In a previous paper on primary carcinoma of the stomach in mice (27) we collected records of four reported cases of squamous cell carcinoma arising in the cardiac portion of the stomach to which we added three observed in this laboratory. Since that publication we have observed one more case (24367). This was an old female mouse that for some time before death had been isolated because suffering from tape worm infection. The stomach wall in the cardiac portion was much thickened and small white nodules were scattered over the outer surface. No metastases could be found. In the liver was an encysted cestode, and there was a prolapsed rectum showing much thickening of the wall. Microscopically the nodules in the stomach are of squamous cell carcinoma, apparently still quite early but of typical structure. One small nodule of similar structure is found in the omentum. The rectum shows a marked overgrowth of squamous epithelial plugs on the outer, ulcerated surface of the bowel, and with many of these an infiltrative character is suggested, but apparently this lesion cannot be diagnosed as carcinoma.

Another new case of carcinoma of the stomach (25911) is more difficult to classify. A female Peromyscus mouse, nearly four and a half years old, which had been isolated all her life and never bred, refused food for several days before death. At autopsy there was found a marked thickening of the entire stomach wall, particularly at the cardiac end where it seemed nearly solid from wall to wall. A thick white nodular outgrowth 6 by 4 by 4 mm. of similar tissue bound together the stomach, liver, and esophagus, while another nodule 8 by 4 by 4 mm. bound together the pancreas and intestinal mesentery posterior to the stomach. No other metastases were found. Microscopically this growth is composed of groups of strands of infiltrating epithelial cells, which are not hornified, neither do they form tubules. They grow profusely throughout all coats of the stomach, sometimes forming sheaths about the tubules of the cardiac portion. The nodules outside the stomach are secondary growths of identically the same structure in lymph nodes. It is not possible to be sure whether this carcinoma is derived from the columnar or the stratified epithelial portions of the stomach. While the gross appearances suggested an origin in the cardia, which is the site of all but one of the recorded cases of gastric carcinoma in mice, yet all of these were frankly hornifying squamous cell carcinomas.

The full details of the three other cases of squamous cell carcinoma occurring in this series are published with illustrations in our previous communication (27) and need not be repeated here. To recapitulate them briefly they were as follows:

No. 5802, male two years old. Squamous cell carcinoma of pyloric portion of the stomach, measuring 15 by 15 by 12 mm. A secondary growth 11 by 12 mm. in the mesentery. No other metastases. Some infiltration of the pancreas.

No. 7851. Male, twenty-five months old. Cardiac portion of the stomach is ulcerated and thickened, forming a mass 16 by 12 by 8 mm. No metastasis. Structure, typical squamous carcinoma, infiltrating all coats of the stomach, and invading the adhesions between the stomach and liver.

No. 16440. Female, aged twenty-seven months. At the junction of the cardiac and pyloric portions a thickened mass from 3 to 15 mm. wide surrounds the stomach. Nodules were found in the omentum and mesentery, metastases in lymph nodes. Structure, typical squamous cell carcinoma with some hornification.

In the same paper was published with illustrations the report of a case of squamous cell carcinoma arising in the metaplastic epithelium covering a prolapsed rectum (8345). This was a male mouse, which had had a prolapsed rectum for six months before its death. The growth was not extensive but seemed to be typically squamous cell carcinoma arising in metaplastic epithelium. Since then we have observed a second similar case.

No. 20052. Female, had a prolapsed rectum for four months before death, which was from hypertrophy of the heart and pulmonary edema. The surface of the prolapse became much ulcerated and after a time showed what seemed to be a proliferation of epithelium. The regional lymph nodes were somewhat enlarged. Microscopically the external surface of the prolapsed bowel shows, in addition to considerable ulceration, areas of definite squamous cell carcinoma, with strands of cancer cells infiltrating through a thick layer of granulation tissue down to but not into the muscularis. Apparently this has arisen from what was part of the cutaneous surface of the anus, and not from metaplastic mucosa as in the previous case. As a possible factor in the inciting irritation, a small piece of wood was found imbedded in the bowel wall, surrounded by granulation tissue and cancer cells.

CARCINOMA OF THE VULVA AND VAGINA

No cases of squamous cell carcinoma of the uterus have as yet been described in mice, so far as we can learn. Although we have found a few fibromyomas and sarcomas of the uterus, we have met with but one epithelial neoplasm, which was an adenocarcinoma.

Erdheim (23) and Haaland (20) each have described a recurrent verrucous growth of the vulva, and the latter a mixed squamous and sebaceous cell carcinoma of the vulva. Two cases of squamous carcinoma of the vulva have been observed in this series and are included among the 70 squamous cell skin carcinomas. (1) No. 7950. This occurred as a condylomatous growth arising distinctly in the vulva of an old mouse, infiltrating the subcutaneous tissues, and was an unquestionably malignant squamous cell growth microscopically. There was in addition metastatic growth in two lymph nodes in the groin. (2) No. 18928. A growth of a warty character and ulcerating slightly developed on the vulva some time before the death of the mouse, spreading about the rectum before death. At autopsy there was found in addition an enlarged lymph node attached to the right ureter. Microscopically the external growth is a typical squamous cell carcinoma with much hornification, and not very marked tendency to infiltration. The nodule attached to the ureter was a lymph node containing a cyst lined with squamous epithelium, or, to describe it better, an epithelial cyst covered with a thin layer of lymphoid tissue. The epithelial wall of the cyst is thin, shows no evidence of infiltrative or other malignant character, and hence does not at all resemble a metastatic growth, but it is difficult to explain the presence of such a structure in this location on any other basis.

In addition to these two carcinomas we have observed an excellent case of carcinoma of the vagina.

Carcinoma of vagina (no. 22582). This mouse was found, nearly a month before its death, with a 3 mm. pink nodule protruding from the vagina. At the time of its death from pneumonia it presented an ulcerating mass 15 by 12 by 10 mm., about one-half of which protruded from the vulva. It seemed to arise from the vaginal wall, the uterus and bladder not being involved, but it ulcerated into the rectum and was much infected and ulcerated. No enlarged glands or other evidences of metastasis could be found. Microscopically the growth is composed mostly of loose masses of keratinized scales exfoliated from the underlying growth, which infiltrates the vaginal wall as a typical squamous cell carcinoma.

KERATINIZING TUMORS OF THE LUNG

As mentioned in our review of the literature, Haaland has described one case of primary keratinizing growth in the lung, and Tyzzer says that he has had "several cases." The influence of heredity on the incidence of tumors of special types is suggested by the fact that of several hundred cases of primary lung tumors in the Slye stock, but one has been definitely found to show keratinization.

No. 13314. A male, age twenty months, which showed no other autopsy findings of interest, had the lower lobe of the left lung nearly replaced by a yellowish mass, which was distinguished from the ordinary papillary adenoma of the lung chiefly by its color. This made it resemble an abscess, but it was much too hard to be an abscess, and there was no pleural exudate or adhesion. Microscopically the tumor consists chiefly of a mass of hornified scales heaped up in waving, concentric layers. Only at the very periphery are living cells found. Here is a narrow growing border of stratified epithelial cells, differing in no essentials from that seen in epitheliomas of the skin. There is no marked tendency to infiltration, the growth apparently progressing by expansion, but the presence of occasional mitotic figures is noted. About the growth there is much round and spindle cell proliferation and numerous foreign body giant cells are found. Our specimen differs from the one illustrated by Tyzzer in having a smaller proportion of living cells. We are not certain whether this growth represents a true neoplasm or a progressive metaplasia due to some persisting chronic inflammatory condition, but the former seems more probable.

Two other mice have shown somewhat related pulmonary conditions.

No. 10561. This mouse, which had also a carcinoma of the skin, had a benign adenomatous growth in the lung which showed some tendency to stratification, but without keratinization.

No. 25136. A male mouse had in the right upper lobe of the lung a mass 14 by 12 by 10 mm. resembling in appearance a malignant tumor of the lung. Microscopically this tissue resembles much more closely an unresolved organizing and necrotizing pneumonia, in which are two irregular, independent areas composed of masses of keratinized scales, with a slender border of flattened epithelial cells. It is quite impossible to decide whether this is a true tumor, or whether it is the cause or the result of the pneumonic condition, although the epithelial growth has the appearance of being much older than the pneumonic process.

TUMORS OF THE MEIBOMIAN GLAND

Two mice have presented growths arising in the eyelids, which, according to their structure, seem to be adenomas arising in the Meibomian glands.

No. 18099. A small, slowly growing mass developed beneath the left eye of a female mouse; from the eye exuded a small amount of thick white exudate. The growth had reached a diameter of 10 mm. when the mouse died from an acute lung infection, and showed no evidence of infiltration or ulceration. Microscopically the growth is composed of papillary structures covered with many layers of epithelial cells. It differs from the normal Meibomian gland in the exaggeration and lawless arrangement of the structures, and the greatly increased number of epithelial cells covering the stroma. The diagnosis of benign adenoma of the Meibomian gland seems justified, especially in view of the size of the growth and the findings in the next case.

No. 27929. An old female mouse, which died of chronic nephritis, had a soft mass, 6 mm. in diameter, beneath the left eye. The gross appearance suggested an epithelioma. Microscopically this tumor is quite the same as the one described immediately above, except for the important fact that it infiltrates down to the bone of the orbit, thus indicating that it is a malignant infiltrating adenocarcinoma.

SEBACEOUS GLAND ADENOCARCINOMA

Such tumors have been described in mice by Murray, Tyzzer, and Haaland, the last two having transplanted them successfully. We have found one case of sebaceous adenocarcinoma of the preputial gland, which closely resembles the growth described and illustrated by Haaland (20).

No. 19895. Beginning at the base of the penis is a mass 30 by 25 by 25 mm. extending well into the inguinal region. The penis was completely imbedded in the tumor, the older portions of which were softened, but about the periphery were hard nodules of newer growth. The testicles and epididymis were not involved. Death resulted from chronic nephritis. There were no metastases. Microscopically this tumor (fig. 12) reproduces closely the normal structure of the preputial gland, but not infrequent infiltration of the stroma by strands of epithelial cells corroborates the gross evidences of malignancy. It corresponds perfectly to the illustration given by Haaland, who also comments on the close resemblance to the normal gland in spite of the definitely malignant character.

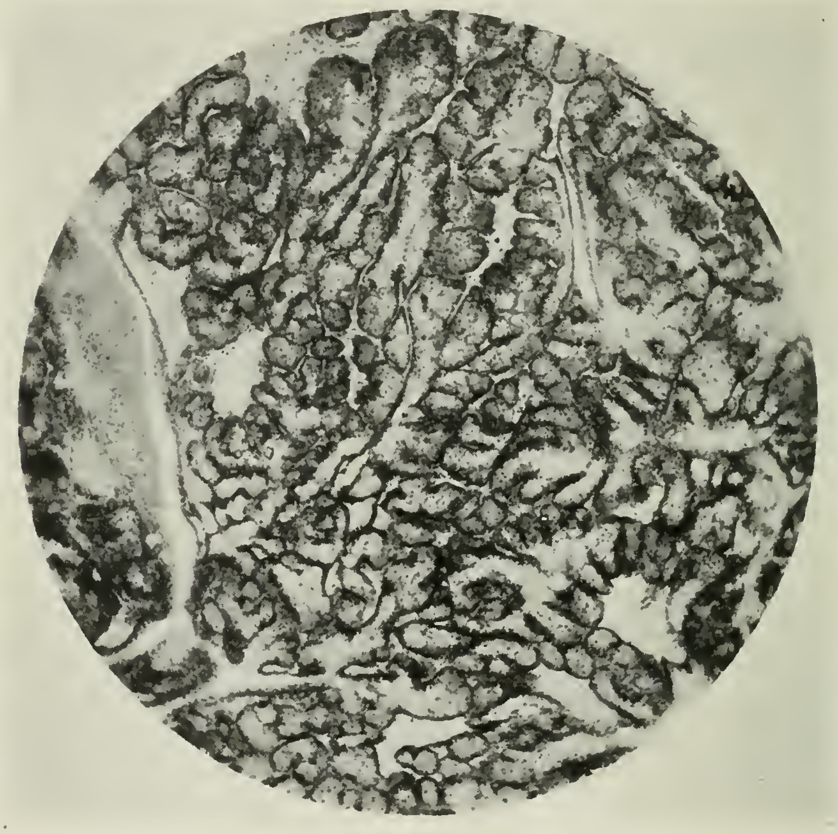

Fig. 12. Sebaceous Adeno-carcinoma of Preputial Gland

This was a large tumor with distinctly malignant characteristics, infiltrating the adjacent tissues, despite its close resemblance to the normal structure of the gland from which it arose. No. 10895. × 60.

SUMMARY

Among 28,000 mice dying natural deaths at all ages, and carefully autopsied, have been observed 153 growths of stratified and squamous epithelium that correspond by the usual standards to true neoplasms, excluding a considerable number of epithelial growths which lack positive conclusive evidence of neoplastic character, although possibly some of these are also early carcinomas. Seventy-one are examples of squamous cell carcinoma of the skin or mouth. They differ from the human skin carcinoma chiefly in a low incidence of metastasis. Fifteen others are of basal cell character, arose always about the head, and produced no metastases. In both these groups the incidence is higher in the females than in the males. Trauma and chronic irritation seem to play an important part in the production of skin carcinoma in mice, most of our cases occurring about the head and face, often recognizably at the site of wounds, and nearly all the skin carcinomas of the trunk arose in old scars. Skin cancers occur at a greater average age than other tumors in mice.

Fifty-six examples of squamous cell keratinizing growths arising in the mammary gland were observed, predominatingly adenocarcinomas with localized areas of keratinization. These also seldom produce squamous cell metastases.

Other tumors in this group were: Four squamous cell carcinomas of the stomach, two arising in the prolapsed rectum, two in the vulva, one keratinizing tumor of the lung, one sebaceous adenocarcinoma of the preputial gland, and, as hitherto undescribed mouse tumors, one squamous carcinoma of the vagina and two adenomas of the Meibomian glands, one of these being infiltrative and apparently malignant.

The literature of the comparative pathology of squamous cell carcinoma in animals is reviewed, and it is worthy of comment that as yet no cases of squamous cell carcinoma of the uterus, bladder, or esophagus seem to have been described in mice.

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PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH

THIRTEENTH ANNUAL MEETING

Held in New York, April 1, 1920

1. Report of the Council

The meeting of the Council was held at Dr. Wood's apartment in New York City, on the evening of Wednesday, March 31, 1920.

The following members were present: Dr. H. Gideon Wells, president; Dr. Robert B. Greenough, vice-president; Dr. Francis C. Wood, and Dr. Willy Meyer, and, by invitation Dr. Frederick Prime. Absent, Dr. E. R. LeCount, Dr. James B. Murphy, and Dr. William H. Woglom.

The report of the treasurer for the year showed a balance on hand of $532.27.

The status of the Journal of Cancer Research was discussed, and a statement from the publishers, showing a total deficit of $110.80 on the four volumes so far published, was read. After some discussion of the proposal, made by the publishers, that the price of the Journal be increased to $6.00 for subscribers not members of the Association, it was voted that no change be made until after the completion of the publication of Volume V.

In order to increase the membership of the Society it was decided that the president should send out a letter to certain men whose names were presented as possible candidates for active and associate membership.

The name of the following applicant came before the Council for election to the Association:

Dr. Michael Levine, Montefiore Home, New York City.

Dr. Wood moved and Dr. Wells seconded the motion that he be elected to membership in the Association.

The resignations of the following members were accepted:

Dr. J. George Adami, Dr. Martha Tracy.

The deaths of Dr. M. J. Herzog and Dr. James Douglas were noted.

Dr. Wood moved that Dr. James Ewing be elected councillor succeeding the retiring councillor. Dr. Greenough seconded this motion.

The following officers were elected by the Council to serve for the ensuing year. Dr. Robert B. Greenough, president; Dr. James B. Murphy, vice-president; Dr. William H. Woglom, secretary and treasurer (re-elected).

The present Council, therefore, with the years of retirement, is as follows:

Dr. E. R. LeCount, 1921
Dr. F. C. Wood, 1922
Dr. James B. Murphy, 1923
Dr. William H. Woglom, 1924
Dr. Robert B. Greenough, 1925
Dr. Willy Meyer, 1926
Dr. James Ewing, 1927

The Council continued in office the present Editorial Board, which is composed as follows:

Dr. William H. Woglom
Columbia University
Dr. Frederick Prime
Columbia University
Dr. Joseph C. Bloodgood
Johns Hopkins University
Dr. Leo Loeb
Washington University
Dr. Ernest E. Tyzzer
Harvard University
Dr. H. Gideon Wells
University of Chicago
Dr. James Ewing
Cornell University

2. A FEW LATE RESULTS AFTER THE RADICAL OPERATION FOR CANCER OF THE BREAST

Dr. Willy Meyer (New York):

SUMMARY

The data given are from the author's private cases which he has followed for the last twenty-six years.

Two radical operations for cancer of the breast have been before the profession since the fall of 1894. Their principal point of difference is the direction in which the surgeon proceeds. One method starts from the chest and works toward the axilla, leaving the clavicular portion of the pectoralis major behind; it requires entering the space between the pectoralis major and minor muscles, the latter usually being divided and then sutured. This method necessarily involves loss of blood. The other method, practised by the author since September 12, 1892, starts from the axilla and works toward the sternum. The tendons of the pectoralis major and minor are divided in the early stage of the operation, necessitating complete excision of both muscles. Blood- and lymph-vessels are primarily divided within the axilla. The lymph-nodes and axillary fat are lifted out in connection with the tumor, before the cancerous breast itself is handled. The entire mass is removed without entering what he calls the "infected area." Hemorrhage is reduced to a minimum.

The final results of the operation from the sternum toward the shoulder, as reported, have been good. Still, small cancerous nodes have been found repeatedly between the pectoralis major and minor muscle, and where cancerous lymphatic nodes have developed, there must be present suspicious lymphatic vessels.

Previous to 1894, excision of the breast for carcinoma was done in two stages, but at the same sitting, first, the removal of the breast with axillary contents; then, the excision of the pectoralis major muscle. This arrangement forced the surgeon to enter widely "the infected area" and caused an unnecessarily great loss of blood. Meyer did not see a single lasting cure after this method of advance. The radical operation changed the results with one stroke, as is shown by a comparison of the author's statistics before and after September, 1894. The first two patients subjected to the modern radical operation were completely cured, and case 4 of this series had for many years enjoyed freedom from cancerous recurrence when she died of old age.

The following patients in the series are alive and well today, from twelve to twenty-five and a half years after operation:

Case 1. Operation in September, 1894; patient thirty-eight years of age (now sixty-four). This was the first case operated upon by the method outlined above. The patient is alive and well today, twenty-five and a half years after operation.

Case 2. Operation in 1895; patient forty-eight years of age (now seventy-three). This woman is alive and well today, twenty-five years after operation.

Case 3. Operation in July, 1902; patient thirty-three years of age (now fifty-one). The patient is alive and well today, eighteen years after operation.

Case 4. Operation in December, 1903; patient thirty-six years of age (now fifty-three). The patient is perfectly healthy and free from recurrence today, seventeen years after operation.

All these patients have full use of the arm and are able to assume the posture of the "Statue of Liberty."

Case 5. Operation in July, 1908; patient thirty-five years of age (now forty-seven). She is perfectly healthy today, twelve years after operation.

Case 6. This patient was operated on in September, 1917, and the case is added merely to show the present line of incision with Handley's addition down to a point midway between the umbilicus and the xyphoid process, for the excision of the fascia covering the upper portion of the recti muscles, in conjunction with the other mass. This addition is recommended because it makes the operation more radical and usually makes possible closure of the wound, without grafting.

Five other patients remained free from recurrence for 4, 6, 8 (2 cases), and 16 years, respectively, and then died of other diseases.

Another patient, a pronounced diabetic at the time of the operation, was well for six years after it, when she succumbed to diabetes, without having developed any signs of a recurrence of the cancer.

Still another patient, operated on for cancer of the right breast in March, 1899, returned in December, 1900, with a carcinoma of the left breast, which was then also extirpated. She was well and free from recurrence when last heard from, in the spring of 1907, six and a quarter years after the second operation.

A few days ago, the author saw a patient now almost eighty years old and in perfect health, who had been operated upon by him for a scirrhus of the breast at the age of seventy-three (seven years ago).

These results, he believes, prove the efficiency of the method; they prove that the *radical operation for cancer of the breast can cure* patients thus afflicted. That not all the patients are saved is due (1) to the stage of the disease in which they reach the surgeon, and (2) to the virulence of the agent that produces the carcinoma.

The importance of the follow-up system was discussed in connection with the collection of statistics on the late results of operation.

Paget's disease (epithelioma of the nipple)

Paget's disease is the most malignant of known forms of cancers of the breast. If ever early and radical operation is imperative, it is in these cases, as will be seen from the following three observations which were made during the last two years.

Case 1. Female, thirty years old, mother of five children, had been in the hands of a quack and had been treated by caustics. When seen in January, 1918, the disease in the breast and axilla had far advanced. After the radical operation the other breast soon became affected, and one year later, also was excised. Then, not long after, the disease became disseminated, and the patient died from cancer en cuirasse.

Case 2. Female, thirty-eight years old, had been in the hands of one of our best x-ray specialists in the city. One and one-half years after cure of the disease of the nipple by radium treatment, there was a local recurrence and a very extensive cancer of the breast, with infected nodes in the axilla and along the subclavian vein. Radical operation was done in December, 1918, followed by renewed x-ray and radium treatment. She now has developed intrathoracic metastases.

Case 3. Male, forty-seven years old, had been in the hands of an experienced surgeon who had extirpated the breast only, without the axillary nodes, evidently because none could be found at that time. One and three-quarter years later, the patient presented a far advanced carcinoma. The radical operation then performed could not save him; and he died from general metastases eight months afterward.

In operating upon mammary carcinoma, Meyer makes it a point to circumcise the skin widely at the base of the breast. He prepares two ample flaps and enfolds them extensively, then divides the fasciae at the base of the two flaps, and extirpates them together with the mass. Involvement of the supraclavicular nodes does not, in his opinion, present a contraindication to operation; on the contrary, he considers it the surgeon's duty to operate when these nodes are infected. Halsted, as well as the late Rodman, have observed patients who remained well for a number of years after the extirpation of these nodes.

In none of the cases presented were the supraclavicular nodes found infected at the time of operation, and hence they were not removed. Meyer has not operated on a single case in which there were no infiltrated axillary nodes.

Meyer believes that statistics regarding the results of the radical operation for cancer of the breast are worthless. They do not prove anything. What *does* determine the fate of the patients is the so-called virulence of the disease. One and the same surgeon may do an equally radical operation in two seemingly early or apparently equally far advanced cases, in one of which the patient may remain well and free from recurrence for, say, twenty-five years, while in the other a regional recurrence and metastases may develop within a few months. All that can be said is that cancer, being a local disease in the beginning, may be cured by a radical operation, if this is done at an early stage.

DISCUSSION

Dr. Robert B. Greenough (Boston); I feel we must acknowledge that surgery of the breast owes much to Dr. Meyer for the work he has been doing, and I am entirely in accord with his reasons for operating in the way which he recommends, namely, beginning the operation in the axilla, and removing the axillary contents, the whole of the breast, both of the pectoral muscles, and the fascia of the chest wall, all in one piece. I feel this hardly needs argument, because it is an accepted principle in operating for the radical cure of cancer in any situation where the anatomical conditions make it possible. I personally have had more experience with the transverse axillary incision than with the incision used by Dr. Meyer, but I think the choice of the incision is a very small matter. It is far more important what is done beneath the skin, and I am entirely in accord with Dr. Meyer in that respect.

In regard to the presentation of statistics in cases operated on for the cure of cancer, some fourteen years ago I had the opportunity of reporting, at the meeting of the American Surgical Association, the results of operations for carcinoma of the breast at the Massachusetts General Hospital, and at the same meeting other papers on the end results of cancer at many different institutions were also presented. Dr. Halsted opened the meeting, and the very first words of his paper were of great significance. He said: "It is especially true of breast cancer that the surgeon interested in furnishing the best statistics may in perfectly honorable ways provide them."[1] I believe that if we are going to attempt a comparison of different methods of operating we must have some definite standard which we accept. I know no better standard than to take all the cases that come into a general hospital under the diagnosis of the disease in question and to record them during the period under observation, with a statement as to what percentage of that number was considered suitable for the attempt at radical cure, and what results were obtained. I am sorry that the idea has not taken hold better in the general reporting of cases. I was looking at a report of cases of cancer of the lip recently published, and in that report all the cases in which an attempt to trace the patient after operation by letter had been unsuccessful, were wiped out of the record. It has been our experience in Boston that the patients from whom one cannot hear are usually those that have actually left this world for another, and I fully believe that patients untraced should be counted as failures. I believe also, as Dr. Meyer stated so emphatically, that one of the great functions of the social service departments of these hospitals should be the tracing of end results. At the Huntington Hospital we have an excellent social service worker who has established a standard in regard to these things, and we do not consider a record closed when the patient has been discharged until we have definite information of the end result. We keep them as live cases until we know that they are dead.

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3. Primary Spontaneous Tumors of the Ovary in Mice—Studies in the Incidence and Inheritability of Spontaneous Tumors in Mice. Fourteenth Report

Miss Maud Slye, Miss Harriet F. Holmes, and *Dr. H. Gideon Wells* (Chicago):

SUMMARY

Among 22,000 mice of the Slye stock dying natural deaths at all ages were 44 with spontaneous primary ovarian tumors, not including simple ovarian cysts. Of these, 38 had simple benign solid papillary adenomas, only occasionally with slight cyst formation; 1 showed a typical papillary cystoma, and 1 a typical solid teratoma containing a great diversity of tissue elements. A second case of this type has since been found in the first 25,000. Of the 38 cases of solid papillary adenomas, 19, or 50 per cent were bilateral, so that there were 57 tumors of this class. There were 4 unquestionable primary malignant tumors of the ovary, all showing the "mesothelioma" type of growth characteristic of malignant tumors derived from the sex glands; one of these produced perirenal metastases. One other tumor of the same type was primary in either the ovary or the adrenal. Two round-cell sarcomas were found, arising either from the ovary or from some other organ, while 2 other sarcomas had produced secondary growths in the ovary. Of the 44 mice with primary ovarian tumors, 26 had tumors in other parts of the body.

In the literature were found reports of eight other cases of benign tumors arising in the ovaries of mice, all exhibiting the same characteristics as the tumors described in this paper.

DISCUSSION

Dr. F. C. Wood (New York): Statistics of this sort are of vital importance in all experimental work in cancer, since they show that if animals are kept to a sufficient old age there is a very large incidence of tumors of all varieties. I think that we who experiment with animal tumors are still supposed by the clinical fraternity in medicine to be working with something which is entirely different from and not in the least comparable to human tumors. But as observations on animals are being extended over larger series of mice, and to other species, as white rats, dogs, guinea-pigs, etc., we are finding instead that tumors absolutely comparable in morphology and biological qualities occur in many of our domesticated animals. This is interesting as furnishing an argument against the current opinion that tumors are a disease of civilization, and primarily of the educated classes, and not a disease which is widespread and generalized through all groups of society. The statistics of the Metropolitan Life Insurance Company, which show a greater incidence of cancer among the laboring classes, point in the same direction, that is, to the fact that there is no immune class. Obviously, in the manual laboring classes the conditions of irritation and of infection (for instance, syphilis, which, while not causing cancer, facilitates its occurrence) result in a larger proportion of tumors in that group, and as we study mice we find that larger numbers of tumors spontaneously appear in these animals. I do not doubt that the same would be found to be true of wild mice if we could keep larger numbers to old age.

Dr. James W. Jobling (New York): I should like to ask Dr. Wells if these ovarian tumors were observed more frequently among the "tumor strains" of mice described by Miss Slye.

Dr. Wells: In reply to Dr. Jobling's question: Miss Slye had hoped to be able to discuss that feature, as, of course, the study of heredity is entirely her part of the work; but on account of illness she was not able to get the material together. We have had strains for twenty-five or thirty generations producing enormous numbers of mice with no tumors. It is perfectly safe to say that mice of certain strains are more likely to have tumors of the ovary than are those of other strains. It is a familiar fact that in the strain of mouse tumor that is used in most laboratories in America, that derived from the Abbie Lathrop stock in Granby, Massachusetts, cancer of the mammary gland is the usual type. Very few cases of tumors of any other tissue have been described in this stock; but Haaland's reports indicate that in the mice in the Imperial Cancer Research Fund in London, tumors of other organs are quite common. I may recall Miss Slye's observations, previously reported, that with tumors of specific organs a very definite relationship to heredity is shown. She reported at one time twenty-eight cases of primary tumor of the testicle, all, with one exception, arising in mice of one definite strain. The exception was in a mouse which had been bitten on the testicle, and which belonged to a strain in which sarcoma was very common. The most striking series which Miss Slye has had was the liver tumor. You may recall that the literature of mouse tumors had previously shown but one case of primary tumor of the liver; since then one or two others have been described. Now Miss Slye has bred, from a mouse with primary tumor of the liver, a strain in which there have developed one hundred cases of primary growth in this organ. While there are no figures on the ovarian tumors, we know that most of them arise in certain strains, though whether strictly within the strains, as in the case of the testicle and liver tumors, we are not yet prepared to say. But in such experiments as these, certain difficulties must be faced. Thus Tyzzer and Haaland have described squamous-cell neoplasms of the lung, a type of which we have seen only one or two examples. Hence minute differences must exist, making one strain liable to one variety of tumor in any given organ, and another liable to a type slightly different.

Dr. C. C. Little (Cold Spring Harbor, N. Y.): How would you explain, on the basis of a single Mendelian factor, the occurrence of these clearly demarcated strains which show specialized types of tumors in particular organs or groups of organs?

Dr. Wells: I am not prepared to discuss that aspect of the work at all; that I leave entirely to Miss Slye. I do not consider myself competent to discuss the matter of genetics in these problems.

Dr. Little: I would like to point out that the result outlined by Dr. Wells is very interesting in support of the theory that the hereditary nature or susceptibility to the occurrence of spontaneous tumors depends on *more than one hereditary factor*. This is evidenced by the clear ability of a particular family to localize its susceptibility to tumor in a particular organ, and is further evidenced by the ability of other families to combine tumors of different organs. Such a result is particularly characteristic of the action of many factors in heredity, rather than of only one. I mention the fact because of the possibility that too much emphasis may be placed on a single factor as the cause of all cancer. The specificity of tissues is so great that it would be surprising if such evidence as that given by Dr. Wells were not found when so careful a study was made.

Dr. William C. Stone (New York): I am very much interested in this presentation because of my own study of ovarian tumors in the human subject. In the first place, as to diagnosis: Dr. Wells spoke of a certain number of these tumors as possibly endotheliomata. Similarly in the human cases, in going over the literature of the so-called Krukenberg tumors, one finds numerous instances in which the picture has been interpreted by many as an endothelioma, by others as a sarcoma, and by still others as a carcinoma. Then, as regards the observation of the occurrence of the tumors in both ovaries, and the difficulty of explaining this in human patients, also, in a large number of instances the ovarian tumors are of secondary nature, and the primary site is elsewhere; but there are numerous cases in which no primary site can be found, and the incidence of the tumor in both ovaries is unexplained. There is one difference, however, if I understand Dr. Wells correctly, between human and mouse tumors. In the majority of cases the so-called Krukenberg tumors in women seem to be secondary in the ovaries. If one considers carefully the descriptions of those reported as primary, one is unable to exclude the possibility that in the majority there was a primary site elsewhere. We found it most frequently in the stomach, gall-bladder, appendix, or some other part of the intestinal tract.

Dr. Wells: Of course, in these cases the histology is not that of the typical Krukenberg tumor. The tumors are bilateral, but they do not resemble the Krukenberg tumor. They do show characteristics which I am quite sure would be cause for a diagnosis of endothelioma or of sarcoma; in fact, before I had studied these tumors carefully I labelled a good many of them endotheliomata of the ovary. It may be interesting to know that every once in a while we see in bovines bilateral tumors of the ovary; some of those I have seen from the stockyards in Chicago are histologically similar to the Krukenberg tumor although nothing is found to indicate a primary tumor elsewhere in the cattle. In mice there have been reported only five or six cases of primary abdominal tumors which could give rise to Krukenberg tumors; so that here we have tumors which are bilateral, and which are distinctly not secondary tumors. Why they should be bilateral in 50 per cent of the cases, and why bilateral tumors are so much more likely to occur in the ovaries I am unable to explain.

4. The Rôle of Neoplasia in Parasitic Diseases of Plants.

Dr. Isaac Levin and *Dr. Michael Levine* (New York):

Previous investigation by the authors on the crown gall have demonstrated that while this condition frequently acts in a manner analogous to animal cancer, the cellular proliferation is primarily a reaction to the invasion of *Bacterium tumefaciens*. The previous experiments were conducted on annuals, biennials, or deciduous trees, in which the period of growth of the host as well as of the crown gall is normally interrupted. In these experiments, some of the galls are benign to the host and behave in a manner more analogous to a scar, a cheloid, than to cancer. In a comparatively small percentage of cases the galls act as true malignant tumors. The parts of the inoculated stem become necrotic above and even below the point of inoculation.

The present investigation was conducted on the rubber tree (*Ficus elastica*), which is an evergreen perennial plant and grows indoors, so that the crown galls may be watched for long periods of time without interference by secondary contaminations. These experiments have shown that twelve months and more after inoculation, nearly every crown gall produces a necrosis of the inoculated branch, though at first the gall may attain a large size without apparent injury to the host-branch. Then frequently, without any additional increase in the size of the gall, the branch becomes necrotic both below and above the gall. This necrosis increases in a centrifugal direction from the gall; thus the tip of the branch may still be alive while a part of the branch below the gall is necrotic.

These phenomena cannot be due to impairment of nutrition since the crown gall frequently attains its largest size without a concomitant necrosis, and the latter is always subsequent; nor can the necrosis be due to the action of some toxic substances produced by the crown-gall cells. It is difficult to conceive of such a rapid change in the metabolic chemical functions of a cell. The most plausible explanation is a change in the reactivity of the host tissue.

For a time the *Bacterium tumefaciens* produces a progressive reaction in the injured and surrounding tissues of the host, which manifests itself in proliferation of cells and formation of a crown. Sooner or later this is followed by regressive reaction which manifests itself in progressive necrosis. Since plants lack the lymphoid tissues and cannot react to parasitic invasion by inflammation, the above described methods of reaction are the only possible ones.

Thus, the neoplasia in the crown-gall disease is primarily a protective reaction of the host tissue to the invasion by *Bacterium tumefaciens*. The morphological studies by the writers of two other parasitic diseases, clubroot of cabbage (*Plasmodiophora brassicæ*), and potato-wart disease, or potato cancer (caused by *Chrysophlyctis endobiotica*) show that identical reactive cell proliferations and formation of new growths may be caused by other parasites besides *Bacterium tumefaciens*.

It is possible that in most parasitic diseases of plants both neoplasia and necrosis take place, though the former may be so insignificant and transitory that it evades detection.

DISCUSSION

Dr. Wells: It is pleasant to hear that potatoes and cabbages contain items of interest besides calories and vitamines. This subject is, of course, of great importance, because the extremely interesting work of Dr. Smith has attracted so much attention, and there has been a lively discussion as to whether these processes in plants are really to be considered as true neoplasms. I wish to express my personal appreciation of this contribution because it must be determined whether these things in plants are true tumors or not, since their interpretation will have much bearing on our evaluation of the work in cancer research.

5. A Phase of Tumor Biology

Dr. Frederick Prime (New York):

SUMMARY

In the past few years much confusion has arisen on account of the various results reached by different cancer investigators doing practically the same line of research. A great deal of this confusion is probably due to the use of tumors whose biological characteristics are really unknown to the investigator. Applications are made to this laboratory every year by workers on the cancer problem who desire a carcinoma or sarcoma which they wish to propagate for a short time in order to carry out some investigation. They then report their results on a few dozen animals, whereas if the characteristic action of the tumor over numerous generations had been known to them, or if they had used a larger number of animals their results would have been very different. For instance, the Jensen rat sarcoma is reported in one paper to take in almost 100 per cent of animals inoculated. In running back over our books for the last six years we find that in 5400 animals surviving at the end of three weeks we have only one series in which there was 100 per cent of takes, and only a few in which the takes were as high as 90 per cent. If our experiment had been done in March, 1914, for instance, we should have had 100 per cent takes in our controls, whereas if it had been done in July, 1916, we should have had only 20 per cent positives. In another instance important conclusions were drawn from two series of tumor 63, one with a high percentage of takes, the other with no takes, but our books show that the same fluctuation has occurred in routine transplantation. The results vary from month to month and from year to year, but if the averages for the months are made the variations are less marked. Of all our tumors, the mouse sarcoma Crocker Fund No. 180 gives the highest percentage of takes and is the most consistent, the number of takes never having fallen below 85 per cent. The Ehrlich mouse sarcoma is a close second to this and in only one month did this fall below 80 per cent of takes.

Certain members of our staff, I myself among them, have had the general impression that our tumors grew less well during the summer months, but upon analysis this proved to be quite incorrect; the death rate among the animals may have been higher, but the tumor growth rate did not show any such change. For six years the Jensen rat sarcoma had its highest number of takes in August, and its lowest in July. The Flexner rat carcinoma had the highest number of takes in April, and the lowest in November. It behooves us, therefore, to know and study our tumors carefully before starting any investigations on them, and to recognize the fallacy of using small numbers of animals in arriving at a conclusion.

DISCUSSION

Dr. S. R. Benedict (New York): I think it would be interesting if Dr. Prime could give some more definite statements as to how much these fluctuations would influence conclusions, that is, whether the results would be diametrically opposed through an increased number of animals, and whether he would recommend that an experiment be based on 5000, or on 500, or on 50 animals. A general criticism of this type calling attention to the number of animals might be misinterpreted unless it is made more specific. In the matter of transplanting a given tumor, for instance, we have quite exact data, and if we transplant from an experimental animal, using the same tumor, we should get a similar growth in both series, except for the experimental factor.

I should like to ask how large a series would have to be, and whether it must be repeated for several months before definite conclusions can be drawn.

Dr. Prime: I think the criticism is exceedingly pertinent. We feel that a good deal of confusion arises with men working along the same line who report such varying results from exactly the same tumor, because they do not quite know the biology of the tumors. One may report results with a certain series or a certain tumor and another give data of an opposite nature, but that does not necessarily mean that one or the other is incorrect. If a little more were known about that tumor, it would probably be found that neither was making very great errors. I think one advantage of a laboratory of this kind is that we have tumors in such large series, and that our records are always available, so that anyone can refer to a tumor with which he is working. But so many of the people who come here for tumors do not care what they get or what happens to it. They want a tumor they can grow in one or two generations. I do not think conclusions should be drawn from experiments with 25 or 50 animals. I think each series should contain one or two hundred animals, and that one series alone should not be taken as a basis for conclusions. As you see here our results vary from month to month; and if experiments were made on several series and the results averaged, I think they would be very much more valuable than are deductions drawn from one or two small groups, as is often done. It is simply that we want to avoid the error of making too sweeping conclusions from too few animals and from a lack of knowledge of the growth rates of these tumors. I think that eventually if experiments are made in this country with tumors whose growth and behavior is known, and with animals whose behavior is known, results from the various laboratories would be very much more consistent.

Dr. Wood: The question has other phases, also, such as the statistical study of tumor regression after taking and the study of the tumor growth rate. In some instances the number of takes is most important, for instance, when attempting to establish immunity against a tumor it is vital that we know that the tumor is not one which establishes immunity against itself. Some workers have reported experiments in which they claimed to have established immunity by means of treatment of the animals, but they overlooked the fact that the tumor used was one which immunizes against itself. In experiments in which attempts are made to influence the growth of a tumor by therapeutic means, it is necessary to use a tumor in which the number of spontaneous disappearances is negligible. Our no. 180 is such a tumor, and because of this fact and because of its easy inoculability, we usually supply it to those who wish to make therapeutic tests. One naïve experimenter, after working with this tumor, wrote that he wished we would send him a better one, because although the tumor he had previously used was curable by his treatment, this no. 180 was worthless because it was too resistant. The value of his cancer cure is evident.

As to the number of animals to be employed: we are in the habit of using small series of animals over a long period of time; instead, for instance, of using three hundred animals for an experiment, and one to three hundred as controls we use two sets of twenty-five animals each, and then repeat the experiment several times, employing a variety of types of tumor, both carcinoma and sarcoma. We thus get long series which can be compared with each other. A few months ago there was published a statement concerning certain effects of x-ray, the conclusions being based on the fact that in twenty or twenty-five animals, treated in one way, there were no tumor takes, while in the control animals there were 80 to 90 per cent of takes. It was, therefore, assumed that an immunity was proved. All it was necessary to do to show that the point was not proved was to open one of our record books of the tumor this experimenter was using and find a page on which were listed twenty-four animals which had been inoculated with the same tumor on the same day, without a single take, while the next lot of twenty-four under the same conditions showed 60 to 70 per cent of takes. The only way to avoid these errors is to repeat the experiment time after time, not necessarily with a large number of animals, and in this way eliminate random fluctuations of which we know nothing, but which are due to the fact, too often forgotten, that an animal is not a test-tube, and that the tumor graft, also, varies in its biological activities.

As to the question whether or not we can stimulate or retard the growth rate of a tumor, the evidence which we have accumulated proves that it is impossible to draw any accurate conclusions as to rate of growth. Some 3000 grafts of tumor no. 180 were planted in healthy mice from one dealer, allowed to grow for three weeks, and then excised and weighed. It was assumed that the average weight of these tumors would furnish a useful standard, yet in the next series of 200 tumors the average weight was found to be double that of the previous 3000. When precautions are taken to have the site of inoculation, the strain, the weight and the age of the animals, and the method of feeding the same in all cases, and yet great fluctuations occur, we are justified in saying that no conclusions as to growth rate can be drawn. As the result of experiments in which only a small number of animals was used and no controls were kept, some observers have claimed to have stimulated tumor growth by chemicals; but while such claims may be correct, they are absolutely unproved because there are no available measurements by which growth rate can be determined.

These are some of the complications of this tumor work, and the only way to eliminate them is to know the biological qualities of the tumor we are using, and to adjust the numbers of animals used to the conditions of the experiment.

Dr. Stone: It seems to me that the purpose of this presentation is an exceedingly good one. It brings to my mind the experience we are repeatedly having at the Memorial Hospital in regard to radium. Men go to Pittsburgh, for example, and make a contract for radium. Then they come to the Memorial Hospital to learn how to use it; and after a few days they return to Pittsburgh and get their radium. The conclusions drawn from their work certainly can be no more accurate than those which are drawn from the observation of the effects of therapeutic measures on a few animal tumors.

6. The Influence of Certain Diets upon Tumor Susceptibility and Growth in Albino Rats

Dr. K. Sugiura and *Dr. Stanley R. Benedict* (New York):

SUMMARY

Curves constructed so as to show the linear and the percentage growth of the fetus and of the Flexner-Jobling rat carcinoma in normally fed animals indicate that there is quite close agreement in growth between the two (the carcinoma and the fetus).

The experiments reported were designed to determine whether general differences in diet influence tumor growth independent of special deficiencies, and to study the effect of certain specific deficiencies in diet upon tumor susceptibility and growth. A complete diet, composed of banana, 83 per cent, yeast, 0.5 per cent, and protein-free milk, 0.5 per cent, was found to yield the same percentage of successful inoculations and the same rate of growth as was secured with a diet of wheat bread and whole milk. The elimination of certain accessory factors in the banana diet was found not to influence tumor growth except when the diet was restricted wholly to bananas. In this latter case, the percentage of successful inoculations was the same as in control animals, but the rate of growth of the tumor was markedly retarded. Such dwarfed tumors showed no histological changes from the normal, and resumed normal growth when the animal was placed upon a normal diet.

DISCUSSION

Dr. Wells: Dr. Benedict's paper giving the curves of fetus and tumor growths recalls a discussion which I heard when a paper from Ehrlich's laboratory was presented. Some German with a mathematical turn of mind had figured the rate at which tumor cells grow. At the end of a certain number of days, he said, one would have enough tumor to inoculate ten more animals, and at the end of three years the total tumor tissue would have formed a cube so large that it would take a ray of light 105 years to pass one side of it. Some of the biologists present said there was nothing remarkable in that, because fetal tissues had been demonstrated to have a fully equal capacity for growth.

Dr. Wood: I did not know when I spoke of tumor growth rates that Dr. Benedict was going to present anything which turned upon the details of growth rate, and I still hold the same views that I expressed a few minutes ago. My opinion of Dr. Benedict's experiments is that they may be right and they may be wrong. The only way to prove them right is to take the two significant series and repeat them on a group of twenty-five rats, some ten times. This will eliminate random fluctuations. Then if the averages show a striking difference between the dieted and the control groups the fact may be accepted that diet influences the type of tumor used, though not any other tumor, and especially not a primary tumor. I can take from my own record books pages that show very much diminished tumor growth rates running over a period of two or three months, followed by a period of very abundant growth, although the animals were untreated. I cannot say, therefore, that Dr. Benedict is right or is wrong and the only way to get proof either way is to repeat the experiment under a great variety of conditions. It is the same problem as the question whether radium does or does not stimulate a tumor. Many workers believe that threshold doses of radium do stimulate but I have been unable to prove it.

7. THE EFFECT OF COMBINED HEAT AND RADIATION UPON TRANSPLANTED ANIMAL TUMORS

Dr. George L. Rohdenburg and *Dr. Frederick Prime* (New York):

SUMMARY

The investigations of Loeb, Stevenson, and others have shown that neoplastic cells are killed in relatively short periods by comparatively low degrees of heat. In the present experiments mice and rat tumors were cut into small fragments suitable for inoculation, exposed to varying degrees of heat for varying periods while in Ringer's solution in a water-bath, and then inoculated into animals, the lethal effect of the manipulation being indicated by the percentages of takes in the inoculated animals. In this fashion exposures to 40, 41, 42, 43, 44, 45, and 46°C. were made for 15, 45, 75, 135, and 195 minutes. The lethal effect of heat was manifested first with 40°C. at the end of 195 minutes, and with 41°C. at the end of 75 minutes; with 44° there were only 35 per cent of takes at the end of 45 minutes.

Bovie has shown that albumins which have been radiated coagulate at much lower temperatures than those not radiated, and it was thought it might be possible to apply this principle to the killing of the cancer cell. Fragments of tumor prepared as were the fragments used in the heat experiments were exposed to x-ray for periods varying from 10 to 30 minutes, 30 minutes representing three erythema doses. Directly after radiation they were exposed to varying degrees of heat after the method outlined in the previous paragraph and then inoculated into animals. It was found that whereas heat alone at a given temperature was not lethal, and x-ray in a given dosage was also not lethal, the two when combined were lethal.

In another series of experiments the process was reversed, heat being applied first, and radiation afterwards. The same effect was noted, the sequence being apparently immaterial.

These results suggest a new method for treatment in human cases, and experiments are now under way in which a practical application of the principle is made on both human and spontaneous tumor animal material.

DISCUSSION

Dr. Greenough: We have felt very strongly that the observation of Dr. Bovie in regard to heat sensitization was one of possibly very great clinical importance, but that further work should be done along experimental lines before any serious attempt is made to apply it to living patients. The point of the greatest importance is that a degree of heat which under ordinary circumstances can be withstood perfectly well by the individual cell results in the death of that cell if it is applied after radiation. The temperatures which Dr. Rohdenburg has indicated on the charts apparently are such that a definite effect can be obtained in a certain small percentage of cases from the temperature alone; to that extent, therefore, the experiment is not quite comparable to Dr. Bovie's original observation; but I am very much interested in the results, and I feel that without question this opens up a field for clinical application in the actual treatment of cases.

Dr. Wood: I think everyone realizes now that we have reached a point where we can say definitely whether or not radium and x-ray will cure any malignant tumor. The only question is will the patient survive the dose. In other words, we have come to a point where it is a question of operative mortality as compared to x-ray or radium mortality. It is perfectly possible to obtain the absolute destruction of cancer cells by a sufficient quantity of x-ray; and the same thing can be done with radium. The only question is the practical application. In my opinion, at the present time most internal tumors require so much radiation that serious, very often fatal, damage to the normal organs would inevitably be inflicted. For example, the lethal dose to a carcinoma cell of a tumor 10 cm. below the skin would be between sixteen to twenty erythema doses, if a highly filtered x-ray were used. I have seen quite serious general disturbances to the intestinal tract result from two erythema doses. The recent German literature, also, contains reports of a number of deaths, some apparently from intestinal obstruction, following heavy doses of x-ray. The problem is to kill every cancer cell, although it is recognized that much benefit can be obtained by the destruction of the main portion of the tumor, the central areas, for example, which are poorly vascularized and easily subject to necrosis from thrombosis of the capillaries of the tumor. But the destruction of the peripheral portions of the tumor, which are well vascularized, is quite another matter. Hence, a very large dosage is required to kill absolutely all the tumor cells, either in the test-tube or in the animal itself. Thus, although a point has now been reached where we can say definitely that cancer can be cured by radiation, with the dosage determined experimentally in this laboratory, the question still is how we can also save the patient's life. With this point kept clearly in mind Dr. Rohdenburg started his experiments. The original experiments Dr. Prime and I began some four or five years ago. The lethal death-points for cells were determined and are standard, so there can be no difficulty in reproducing these results anywhere if tumor 180 is used. An x-ray machine can be calibrated biologically. My clinical experience shows that in a rapidly growing carcinoma in man the death point is about the same as in 180. The slow growing squamous-cell epitheliomata seem to have extra resistance, owing to the ability of the cells to cornify and practically to enter a resting stage. It is now accepted that the lymphosarcoma and many of the bone tumors are more susceptible to x-ray than are the very rapidly growing highly malignant carcinoma or sarcoma. The maximum practical dose of x-ray or radium which the patient can survive remains to be determined, but if it is remembered that there is, after all, a surgical mortality and that untreated cancer is fatal, we are justified in giving radiation pretty close to the limits. Most radiation treatments are far beneath the killing dose for the cells of most of the internal tumors. I do not believe that the induced connective tissue reaction around the tumor cells kills them. It may encapsulate them for a while, but usually the cell enclosed with scar tissue finally begins to grow and destroys the patient. After having given the maximum dose of x-ray which the skin of the patient can stand, we might be able by thermo-electrical devices to apply heat which does not seriously damage the skin and which produces death of the cells. The morphology of cells killed by heat is practically the same as that of cells killed by x-ray or radium, and I think that this paper is important from a therapeutic point of view, as well as a scientific one. It may be possible by some high frequency method to add to the semi-lethal dose of x-rays a semi-lethal dose of heat without causing the death of the patient and that, I think, these experiments clearly show. The practical application will require long careful study on human beings.

Dr. William Duane (Boston): In regard to the practical application of heat and x-rays, this was tried very extensively in Paris seven years ago, and there was claimed a good deal for the combination. There was one difference between that procedure and the one reported here, the difference being that the two destructive agents were applied at the same time. Keating-Hart warmed the human tumor tissues and at the same time applied x-rays, and I understand that here heat was applied either before or after radiation. It would seem probable that the application of the two at one and the same time would produce a greater effect. As regards the ability to destroy any tumor by radiation, if an intense enough radiation is used almost any organic compound can be destroyed. Water can be decomposed by the action of the rays.

Dr. Rohdenburg: I would like to say that these experiments differ from those previously reported in that lower degrees of heat were used over a much longer period. In the recent German literature the combination is reported to have been tried, but tried by combining diathermy produced by seven amperes of current for a period of thirty seconds. This dosage produces coagulation necrosis and a typical burn. Our aim is to obviate this factor of burn. The tumors we have treated with high frequency, using 25 milliamperes per square inch of electrode surface and continuing the application for twenty minutes, show scarcely any recognizable change in the tissue for a period of a week or ten days. This dosage produces a temperature between the electrodes of 40°C., or 104.8°F. which is very readily withstood by any normal tissue, and many degrees below the temperatures produced by Keating-Hart or the more recent investigators.

8. Interstitial Injections of an Active Deposit of Radium Emanation in a Rat Carcinoma

Dr. Halsey J. Bagg (New York):

SUMMARY

In this experiment a definite attempt was made to use an "active deposit" of radium emanation as a local agent, employed in the form of a solution, to control the growth of an experimental rat tumor—the Flexner-Jobling rat carcinoma. The writer gave a preliminary report of the results of treating twenty-one animal tumors.

Interstitial injections of a radio-active salt solution of radium emanation were found to retard materially the growth of the tumors and in some cases to cause their regression, resulting in characteristic cellular changes in the tumor tissue. Definite characteristic, histological radium changes were noted—frequent hyperchromatic and homogeneous nuclei, cellular hydrops, fairly extensive central necrosis, and a terminal onset of fibrosis.

It was found that comparatively large doses of radium could be injected into rat tumors without the escape of the radio-active solution into the surrounding tissues. (A comparatively small intravenous or subcutaneous injection of the same solution invariably results in severe reactions.) The rapid decay of the radium when used in this form, in addition to the walling-off effect of the tumor capsule, appears to be sufficient to confine the radium action to a desired zone of tissue. This was an encouraging observation from the standpoint of human therapy.

DISCUSSION

Dr. Duane: I think it would be well to emphasize the very great danger of this method of using radium or radio-active substance. The alpha rays are utilized, and since of the total activity of all the beta, gamma, and alpha rays, about 90 per cent is due to the alpha radiation, it is evident that the alpha rays produce relatively tremendous effects. It is well to bear in mind, therefore, that this method of treating tumors should be employed with very great care. Over 100 millicuries of deposited activity would be a dangerous dose for any human being.

Dr. Benedict: I should like to ask if this is not simply a question of the size of the tumor mass; that is, one might destroy a small tumor, but affect only the center of a large one, making it necrotic.

Dr. Stone: It seems to me that both of these methods, the intravenous injection of the active deposit and the local infiltration of the tumor, represent a type of experimental work which is most desirable, but, as Dr. Duane has just said, we must use extreme caution in applying this practically. The general constitutional effects from intravenous injections are very marked. So far we do not know the constitutional effects from the infiltration method, because it has not been applied except in two or three instances, and then very cautiously. It does seem, however, as if we might make use of the alpha rays in this infiltration method, which, as Dr. Duane has said, comprises so much of the energy of radium, and which apparently we cannot use in any other way. There is another point in regard to the practical application of this infiltration method. Personally I dislike to think of radium used either by this method or by the surface application to the extent that we get actual destruction or caustic effects. I conceive of using radiation for a strictly biological effect, without causing absolutely local destruction, for years prior to the use of radio-activity all of us had experience with the use of the cautery, and we knew just how much tissue we could destroy with that. With the use of radio-activity we do not know just how extensive the destruction is, how long it is going to continue, or what the destructive effects upon the normal tissues in the neighborhood of the tumor are. The possibilities of destruction are so great that we must use extreme caution. In making a practical application of this infiltration method I think we must begin with the very smallest dose so as to get the so-called biological effect, and not a local caustic effect on the tumor itself.

Dr. William B. Coley (New York): The work of Dr. Bagg is extremely interesting and valuable, but I believe that the cautions given by Dr. Stone and Dr. Duane are timely. At the Memorial Hospital Dr. Janeway has used the method in a considerable number of cases in human beings, beginning with small doses and increasing to fairly good sized ones; but so far none of the results gives us reason to believe that it is likely to be of permanent value as a therapeutic measure. I believe, as Dr. Bagg points out, that the cases in which good results are obtained are those in which the tumor is localized by the surrounding tissues, and if that is so, why can not one use the bare tubes which will remain localized, and not get into the tissues and damage the kidney, liver, etc.? I believe with Dr. Wood that we can always give enough radium to kill the cancer cells, but not in most deep seated cancers without killing or injuring the patient.

Dr. Bagg: Dr. Benedict has brought up the subject of the relation of tumor size to the subsequent reaction to the treatment. I have found that in most cases the central portions of the treated tumors were necrotic, and Dr. Ewing, who has seen my sections, has called my attention to the peculiar manner in which the radio-active solutions have diffused to different parts of the tumor, causing more extensive reactions in certain areas of the tumor than in others. I have tried in some recent work to infiltrate the tumors from within, by first placing the hypodermic needle in the center of the mass and then moving it to different parts of the periphery of the tumor, and injecting a small amount of the solution in each place. In regard to the apparent increase in size of some of the treated tumors, I would say that this is no doubt due to the production of an edematous condition and the accumulation of fluids in the center of the tumor, in which case the ring of tumor tissue was crowded to the periphery, while the actual bulk of the tumor tissue was no greater than the amount present at the beginning of the experiment.

9. Factors Underlying Susceptibility to a Transplantable Tumor in Mice

Dr. C. C. Little:

SUMMARY

A sarcoma, J. W. B., which originated in an inbred race of Japanese waltzing mice grows upon transplantation in 100 per cent of the animals of that race inoculated. It fails to grow progressively in more than 99.5 per cent of the common non-waltzing stock mice inoculated.

Beginning two weeks after inoculation each animal is observed at weekly intervals, and the presence or absence of growth is noted. When a growth is present the animal is recorded as +; when no growth is found, it is recorded as −. Two groups of animals have been observed. They are (1) ordinary non-waltzing stock mice series (N.); (2) back-cross hybrids (B. C.) produced by crossing an F1 generation hybrid between Japanese waltzing and common non-waltzing mice back with the common non-waltzing parent race.

The present series of experiments deals with growth of the tumor from the second to the sixth week after inoculation, inclusive. Many

of the animals showing a growth at the sixth week observation showed eventually regression and disappearance of the tumor. The factors studied are, therefore, those allowing the initial six weeks' growth of the tumor. This growth may or may not be continued later, depending upon the hereditary constitution of the mice used. The animals inoculated were divided by age into ten groups, respectively, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 or more days old at inoculation. For each of these groups the percentage showing growth at 2, 3, 4, 5, and 6 weeks after inoculation was determined. The results of *all* groups according to weeks after inoculation were as follows:

WEEKS	N., PER CENT +	B. C., PER CENT +
2	16.1	27.1
3	13.9	21.0
4	10.7	12.4
5	7.8	13 6
6	5.3	13.1

It will be noted that the percentage of animals showing growth in the N. series steadily decreases, while in the B. C. series, there is an initial decrease followed by a rise to a level at the fourth week at or near 13 per cent. The total per cent for the N. series is 11.12±46 and for the B. C. series is 17.54±83. The difference is 6.42±95 and is, therefore, significant, being 6.7 times its probable error.

Studied by age groups with the sexes combined, there is seen to be a distinct difference between the two groups.

AGE AT INOCULATION	N., PER CENT +	B. C., PER CENT +
days		
2 to 10	12.87±0.6	13.77±1.05
12 to 20+	9.45±0.6	21.58±1.28

While in the N. series the *lower* age group shows the most growth, exactly the opposite is true of the B. C. series in which the higher age group shows a significantly higher percentage of growths.

When the sexes are studied separately the males of both series and of both age groups are apparently not significantly different from each other. The females, on the other hand, show a significantly *lower* percentage of growths in the *higher* age group of the N. series and a significantly *higher percentage* of growths in the *higher* age group of the B. C. series. The higher age group of both sexes is more mature and further differentiated than is the lower age group. Within the higher age groups many of the females become sexually mature during the period of observation. This gives to them an additional chance for differentiation and the assumption of sex limited or other biological characters dependent upon their hereditary make-up and upon the degree of differentiation which their tissues have reached.

AGE AT INOCULATION	N.		B. C.	
	♂	♀	♂	♀
days				
2 to 10	15.70±1.64	19.46±1.31	14.51±1.51	12.12±1.76
12 to 20+	10.30±1.20	9.39±0.87	15.38±1.55	25.74±2.07

What we are observing, therefore, is a racial difference. In the N. series a steady decrease in percentage of growths, especially marked in the females, is found. In the B. C. series there is at first a decrease followed by a rise, a level of about 13 per cent growth being reached. This level is due for the most part to the presence in the B. C. generation of animals which will show permanent progressive growth of the tumor. The results of both series coincide with and amplify the explanation of the hereditary nature of susceptibility to the J. W. B. tumor, advanced by Tyzzer and the writer in 1916.

A STUDY OF A LIPOMYXOSARCOMA WITH COMMENTS UPON THE ORIGIN OF THE FAT CELL

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Neoplasms of mesenchymal origin are frequently the cause of much controversy owing to a lack of differentiation into tissue which would permit their being placed in a definite organoid category. The term "liposarcoma" has been used to denote malignant change in the connective tissue of a lipoma, itself one of the most benign of tumors, and also to accent the fact that the growing fat cells have invaded the surrounding tissues or metastasized. A tumor which presents the accepted criteria of malignancy, derived from fat cells which are atypical in size, shape, and staining characteristics, with many mitotic figures and invasion of septa and capsule, is of the greatest rarity, and a study of such a growth may throw light upon the origin of the fat cell, regarding which there is much conflicting opinion. The writer has lately had the opportunity to examine a tumor of that type, removed from a patient in the Surgical Out-Door Department of the Peter Bent Brigham Hospital, and with the kind permission of Dr. Harvey Cushing a brief summary of the case is given.

The patient, a Russian Jew, came to the hospital on November 18, 1920, complaining of a growth in his right thigh. His past history is unimportant except in so far as it concerns the tumor. In 1917 he noticed a firm swelling the size of a walnut beneath the skin on the mesial surface of the right thigh. It caused no pain. A few months later it was noticeably larger, and massage was instituted for a short time. It continued to grow slowly and became a little painful, particularly in cold weather. He was, however, able to work in his store, and he walked into the hospital.

Physical examination was negative except for the local findings. On the inner surface of the upper third of the right thigh, beneath the skin and extending deep into the muscles, was felt a somewhat movable, irregularly rounded growth, about 18 x 6 cm. in size, and not tender. There were no palpable nodes. On November 19, under novocaine anesthesia, the growth was removed by Dr. A. H. Brewster. It seemed to have three distinct capsules, but there was no evidence of invasion of the surrounding muscles. The outer two capsules were thick and fibrous, and stripped away readily from the more delicate layer covering the tumor. Most of the blood-vessels entered through a sort of pedicle on the deeper portion of the tumor. The dead space was closed with catgut sutures and the skin with silk. On December 21 the wound had entirely healed. On February 3, 1921, the wound was in good condition, with no evidence of local recurrence or metastases, and the patient felt perfectly well.

Pathological examination. The specimen consists of an irregular mass measuring 17 x 8 x 5 cm., and weighing 510 grams. It is surrounded by a delicate transparent capsule beneath which ramify a moderate number of dilated blood-vessels. The larger vessels converge upon a narrow zone on one side which probably represents the pedicle where the vessels were clamped off. It is uniformly firm, more so in slightly depressed areas between the bulging large lobules into which it seems to be divided. A longitudinal incision shows it to consist of two main types of tissue. About three-fourths of the tumor is pale, rather pink, and opaque, with the consistence of a very cellular tumor. The mass is divided into more or less definite lobules by fibrous septa of varying thickness. A broad fibrous band apparently invaded by streaks of tumor separates it into two main parts, which are in turn subdivided into smaller lobules by narrow vascular septa. More or less well circumscribed areas suggesting mucoid degeneration are present in the middle portion, irregular wells of mucilaginous fluid bathing the surface like honey in a comb. The fluid gives the characteristic reactions of true mucin.

Microscopical examination. Tissue was fixed in 10 per cent neutral formalin and in Zenker's fluid. Frozen sections were stained with Scharlach R and hematoxylin, other preparations with methylene blue and eosin, phosphotungstic acid-hematoxylin, aniline blue-acid fuchsin, osmic acid, Mayer's mucicarmine and mucihematein, and Verhoeff's elastic tissue stain. A study of the sections gives the following facts.

The tumor is surrounded by a thin compact layer of connective tissue in which are a moderate number of elastic fibers. The capsule sends prolongations into the tumor dividing it into smaller units. Elastic fibers and thin-walled arteries and veins accompany the septa and many fine capillaries are present in the stroma, which consists of a delicate collagenous reticulum in which the tumor cells lie, and which appears to arise for the most part from the blood-vessels. The tumor is very cellular except in small areas which have undergone mucoid change, where the tissue suggests that of the umbilical cord. In shape the cells vary from medium sized spindle cells through intermediate forms to the more or less round outline of mature fat cells. The nucleus follows the general shape of the cell and has a densely staining nucleolus. Practically all the cells contain refractive fat droplets which stain with Scharlach R and reduce osmic acid. In the spindle cells the droplets are, as a rule small, almost always smaller than the nucleus, and tend to localize in the vicinity of the nucleus. These spindle cells often have delicate wavy processes of some length. In the intermediate, polyhedral forms the fat drops become larger and coalesce, the nucleus stains more deeply, and mitotic figures are seen in large numbers. The dividing cells have a large fat content. The more mature round cells are distended with a single droplet of fat which occupies practically the entire cytoplasmic space and flattens the nucleus, giving the typical signet-ring appearance. Many of these cells have ruptured, and the fat has escaped into the interstices, where it has formed little pools. Invasion of the capsule and septa by the tumor cells has occurred. No intravascular growth is found, however. Scattered through the stroma are also many mast cells, none of which contain oxidase.

Sections from the areas of "mucoid degeneration" show an abundance of loose wide reticulum containing mucin. In this are present a moderate number of tumor cells of all types with large and small fat droplets. With specific stains mucin can be demonstrated in most of the cells in the form of a fine or coarse granular deposit in the cytoplasm, clustered about the fat droplets. Mucin is present in small amount in the cells of various other parts of the tumor but is most abundant where it exists in macroscopic quantities.

DISCUSSION

A lipoma is a very common and benign tumor composed of mature fat cells. A liposarcoma is much less frequently met with and in a large percentage of cases has shown areas of myxomatous degeneration. Knox (1) in 1919 collected twenty-five cases and added two more. The names lipomyxosarcoma, myxoliposarcoma, liposarcoma myxomatodes, and myxosarcoma lipomatodes have been used by various writers, depending upon which tissue was in excess, the fatty or mucoid, or whether the writer regarded the mucous tissue as a product of degeneration of the fatty tissue or vice versa. "Sarcoma" denoted invasion, recurrence, or metastasis. Fibrous tissue, cartilage, and bone have also been found in these neoplasms, making them rather mixed tumors, although with all elements derived from the same germ layer. Hirsch and Wells (2) studied a retroperitoneal tumor of 69 pounds, parts of which were pure lipoma, others fibrosarcoma. In none of the cases described have mitotic figures been found in the fat cells, although evidence of direct division has been noted. The most frequent sites of these tumors are the retroperitoneal tissues, the muscular fasciæ of the thigh and leg, the mesentery, the cheek, and the breast.

The tumor forming the basis of this paper has several unique features. The bulk of it is composed of cells in practically all of which is much fat. Most of these cells are of the embryonal type, being spindle or stellate with delicate fibrillary processes. Many transitional forms are present, up to and including the large round signet-ring mature fat cell. Many cells are in mitosis and these also contain fat droplets. About one-fourth of the tumor is bathed in mucin, and the cells in such places contain both fat and mucin.

In offering an explanation for the presence of mucin and fat in the same cell, we must go back and consider the embryological development of the connective tissues. The connective tissues which include fat and mucous tissue are derived from the mesenchyme. Todd and Bowman (3), in 1845, were among the first to suggest that adipose and areolar tissues are distinct and independent. Kölliker, in 1856, described the original fat cells as glands, such being found in the axillae and mesentery, and around the kidneys. In 1870 Toldt's (4) investigations led him to the conclusion that the "glands" of Kölliker are the source of fat, and he called them "fat organs." Flemming

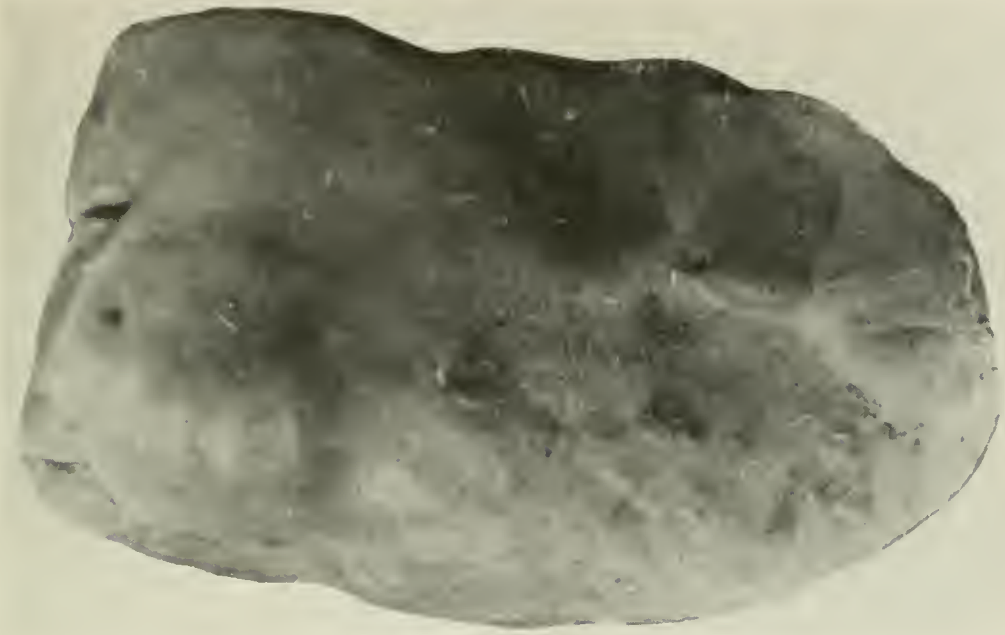

Fig. 1. Photograph of the Original Tumor, Exterior

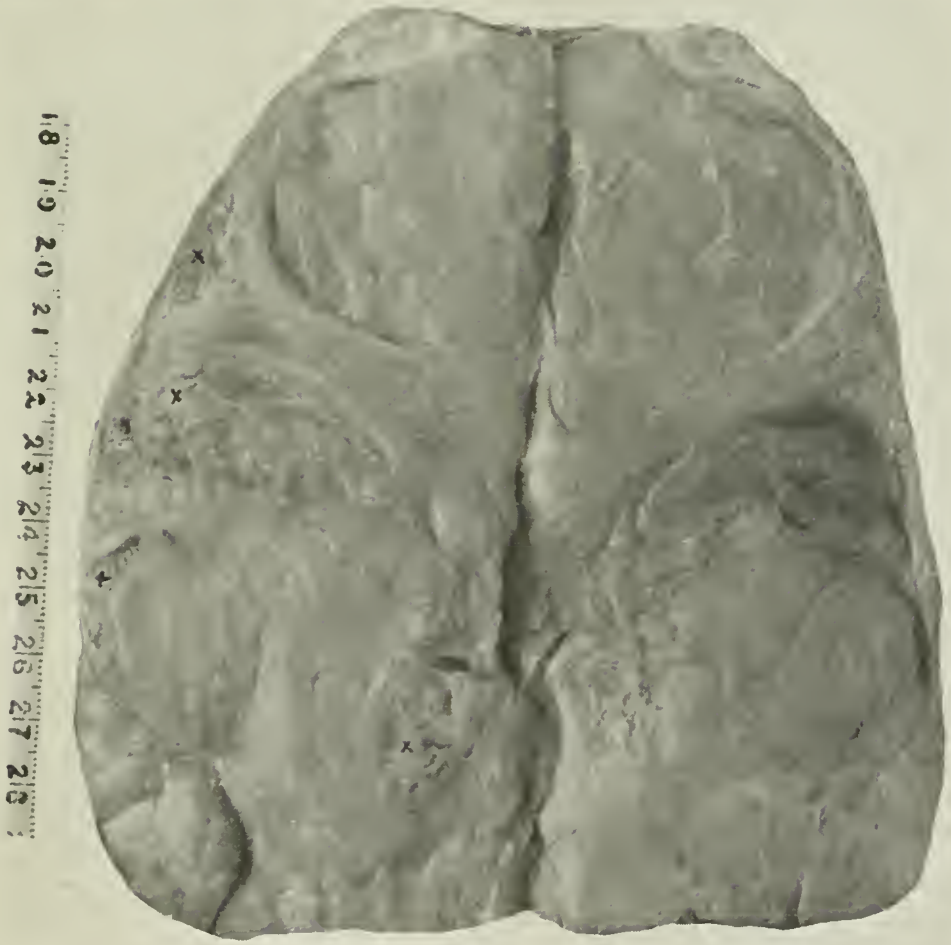

Fig. 2. Photograph of the Tumor, Opened by Longitudinal Incision
The bulk of the tumor is pale pink, opaque and firm. The crosses (×) mark areas of myxomatous tissue.

(5) in 1876, in a comprehensive study, advanced the opinion that the ancestral tissue of fat is a fibrillar connective tissue. Hammar (6) also held this view.

In 1894, Borden (7), in a comparative study of the fat cell, stated that (i) in the lower vertebrates the fat cell is developed from one form of cell arising from special centers; (ii) in the higher vertebrates the fat cell is developed from two forms of cells differing greatly in size and shape; and (iii) one of the original fat cell forms in the higher vertebrates is homologous with the special center forms of the lower vertebrates. This cell is gland-like and in no way resembles the connective-tissue cell; the other form, while closely resembling the connective-tissue cell, is most probably a special cell derived from the special center forms by cell division and "migration metastasis." Multiplication is by indirect division and the cells never turn into connective tissue cells.

Bell (8), working with material from a calf fetus and from steers, described a peculiar open-meshed "pre-adipose" tissue which forms well defined lobules before true fat cells appear. This "pre-adipose" tissue consists of loosely arranged cells with two or more coarse processes. The branched cells may contain fat droplets a long time before they assume the rounded form. Bell supports Flemming in the belief that the pre-adipose tissue is clearly a fibrillar connective tissue. He also described how the cells nearest the blood-vessels filled with fat first, the processes becoming absorbed. Altmann's granules were observed when the cells were yet branched and before the first fat droplets had appeared.

In Lewis and Stöhr's Textbook of Histology (9) it is stated that in the four-month embryo the fat cells are quite like the surrounding fibroblasts, being fusiform or stellate and containing vacuoles or droplets. Mallory (10) emphatically says that the fat cell is a perfectly definite type of cell formed by differentiation from a mesenchymal cell, that it is not a fibroblast, does not arise from one, and, when it undergoes atrophy, does not turn into one.

The earlier writers, in speaking of the connective tissue cell, probably referred to what is now termed the fibroblast. Consequently, at the present time the opinion is strongly held by many observers that the fat cell is essentially a modified fibroblast, whereas others assert that it cannot be so considered. I believe that the demonstration in this tumor of mucin in the cells which otherwise appear as fat cells supplies an argument in favor of the former idea.

Mucin is a slimy substance produced by certain epithelial (beaker) cells and by the fibroblast under special conditions. We are concerned here with its production by the latter cell. The umbilical cord furnishes the classical example of mucous tissue. The mucin is said to be secreted by the fibroblasts without the formation of special granules or vacuoles (Lewis and Stöhr). Cohnheim restricted the term mucin to those substances which are excreted by epithelial cells, and reserved the name mucoid for closely allied bodies which occur in various organs of the body, such as the vitreous humor, cornea, umbilical cord, and tendons.

Mucin has certain characteristic chemical and physical reactions which permit its identification, so that the term mucoid seems unnecessary except for describing gross physical qualities of the fluid. It is not produced normally in the adult, but in tumors of mesenchymal origin it is found not infrequently, as in myxoma, fibroma, chondroma and osteoma, and in the more rapidly growing myxosarcoma. Ewing (11) states that mucous tissue "is genetically related to fat tissue into which it is extensively transformed during normal growth. . . . . It does not appear that true myxomas ever tend to differentiate into fibroma or lipoma." He also asserts that the spindle and star cells in the mucous matrix may contain hydropic or fatty droplets.

The question naturally arises, is the substance in the tumor cells which stains with Mayer's mucin stains really mucin? The tissue in which these cells are found in greatest numbers is bathed in a slimy fluid which gives the accepted tests for mucin and likewise stains with mucicarmine and mucihematein.

It is, of course, impossible to be certain that the intracellular substance is mucin, yet it stains "specifically" and any other interpretation would seem illogical. The cells otherwise have the appearance of young fat cells such as are found in other parts of the tumor.

It might seem unwise to attempt to settle the origin of a cell solely by study of a tumor derived from that type of cell, particularly in view of the frequency of metaplasia in pathological experience. But metaplasia apparently has a very narrow scope, its best illustrations being in epithelial reactions, and application of the principle to the connective tissues has led to great differences in interpretation. To explain the secretion of mucin by the cells of this tumor as an example of metaplasia does not seem as rational as to assume that the cells which are essentially fat cells take on the characteristics of a less differentiated tissue, a noteworthy deviation from the normal being that the mucin is stainable *in* the cells.

These observations, it seems to me, indicate that the fat cell and the fibroblast (considering the mucous connective-tissue cell as a modified fibroblast) are very closely related and give support to the hypothesis that the fat cell is derived from the fibroblast.

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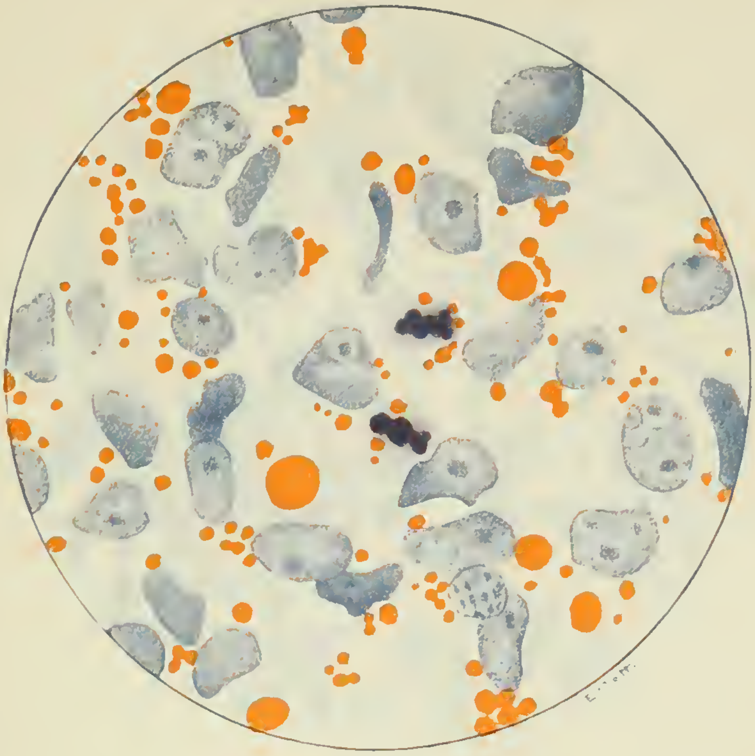
PLATE

PLATE 1

Fig. 3. Drawing of microscopical field, high power. Frozen section stained with Scharlach R and hematoxylin. The distribution of fat in the cells and interstices is shown. In the center are two cells in mitosis and containing fat droplets.

Fig. 4. Drawings of four cells in a section stained with Scharlach R and hematoxylin, showing the transition from spindle cell to the mature signet-ring fat cell.

Fig. 5. Drawings of three cells in a section stained with Mayer's mucicarmine, from an area of myxomatous tissue. The finely granular mucin is seen in the cytoplasm between the fat droplets.



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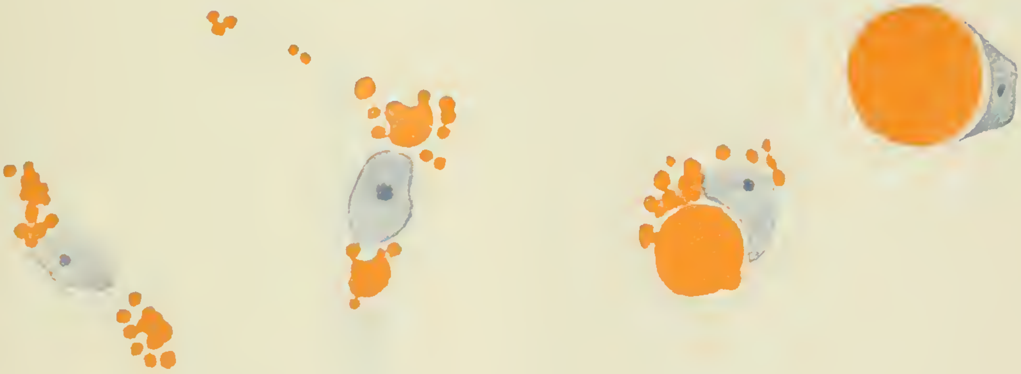

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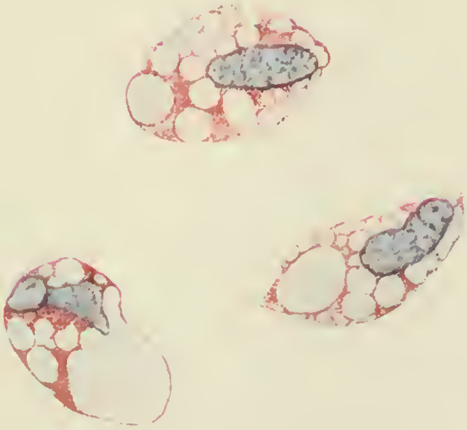

V

PARABIOSIS AND TUMOR GROWTH

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The subject of tumor immunity is of considerable importance, and any approach that promises to throw light upon this as yet unsolved problem should be thoroughly investigated. The effect of parabiosis upon tumor growth has been studied by various investigators, but their results and conclusions have been far from uniform. In an attempt to clear up this matter, the experiments here reported were undertaken.

Sauerbruch and Heyde (1), in a series of experiments with parabiotic animals, noticed that the death of one animal was always followed by the death of the other in a few hours unless the live animal was cut away from its dead partner. This, they maintained, is probably due to the absorption of cadaver toxins from the dead animal. They also noticed that when iodine solution was injected into one animal, the urine of the other gave a positive iodine reaction within forty-five minutes. That corpuscular elements also could pass from the one to the other, they proved by injecting a culture of anthrax bacilli into the right animal and recovering the characteristic organisms from the heart's blood of the left. From their anatomic studies, the authors conclude that the operative wound uniting the two animals heals exactly as does that in a single animal, except that the reaction is more intense, owing probably to "the foreign body" reaction.

Friedberger and Nassetti (2) studying the antibody formation in parabiosis found that agglutinins formed as a result of the injection of typhoid bacilli or Vibrio elbensis into one animal could be recovered from the non-injected one. They also demonstrated that one animal could be immunized passively by the injection of these organisms into the other. Morpurgo (3), in a series of most interesting experiments, showed convincingly that after a bilateral nephrectomy was performed upon one rat, the kidneys of its partner in parabiosis were able to compensate for their loss and maintain life for both animals—in one instance for forty days. In another case, he succeeded in keeping two parabiotic rats alive for seventeen days after unilateral nephrectomy in one animal, the two kidneys of the other having been previously removed. Jehn (4) and Sauerbruch and Heyde obtained similar results in rabbits.

The findings in all these investigations show how very intimate is the biological relationship between the parabiotic animals. Lambert (5) studied the influence of parabiosis upon the growth of transplantable tumors in rats, and concluded that the growth of mouse tumors in rats is definitely promoted by parabiosis in that the percentage of successful inoculations is increased, the rate of growth accelerated, and the duration of active growth extended. Rous (6) in a series of experiments with rats concluded that parabiosis has no effect upon the growth of tumors. Albrecht and Hecht (7), however, showed that parabiosis in mice exerted a distinct retarding influence on the growth of tumors, and explained this phenomenon as similar to the immunizing action of subcutaneous injections of blood, embryo, or normal tissue.

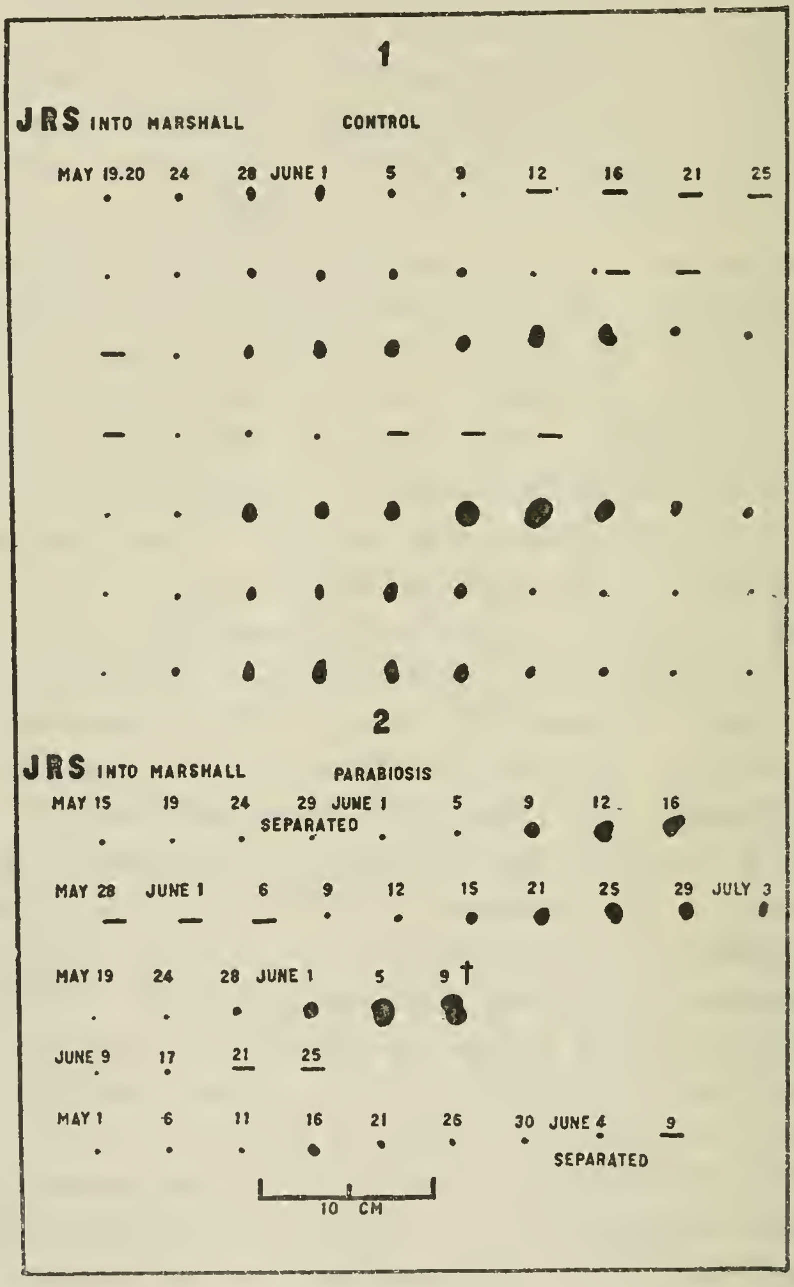
In a previous study (8) the author showed that the intraperitoneal injection of blood from immune animals into susceptible ones was without retarding influence upon the growth of tumors in the latter. This paper is a continuation of the previous study and is based on the following working hypothesis: We assume that an immune animal possesses substances antagonistic to tumor growth, or that it lacks those elements necessary to tumor for its growth. Having proved that these substances do not reside in the circulating blood, we further assume that they exist somewhere else in the organism. By performing a parabiosis between a refractory and a susceptible animal, we bring these two organisms into very intimate biological relationship. We have further assumed that it may be possible

(1) for the "antibodies" in the immune animal to cross over into the non-immune parabiotic partner and confer upon it the property of antagonism to tumor growth; (2) for the substances that make for tumor development in the susceptible animal to cross over into the immune one and render that animal susceptible. To test these possibilities this study was undertaken.

In one part of these experiments, 44 pairs of animals were united in parabiosis. Two distinct breeds of animals were employed—August rats, which are susceptible to the growth of Jensen rat sarcoma (J R S) and Marshall rats which are refractory to this tumor. Five days subsequent to the uniting of two of these animals, the Marshall rat was inoculated with 0.003 gram of Jensen rat sarcoma. Of the 44 pairs, 3 died before the tumor inoculation. The parabiotic life period of the others varied from four to thirty-five days. Five pairs were lost leaving 36 pairs from which to draw conclusions. In only sixteen instances (44 per cent) could tumor formation be made out and at that the majority of these growths were pinhead size. Of the control animals, individual rats of the same breed receiving similar inoculations of Jensen rat sarcoma, 32 out of 43 (74 per cent) showed positive results. These tumors were larger, appeared earlier, and lasted longer than those in the parabiotic series.

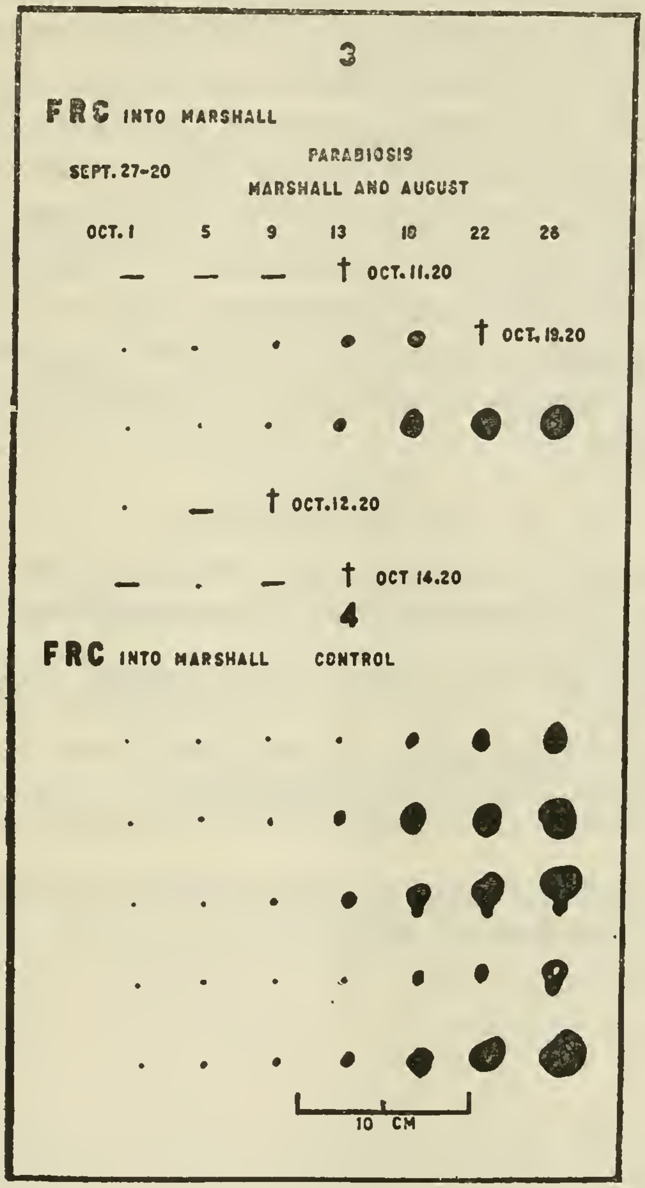
In chart 1 are shown the tracings of the growth of the Jensen rat sarcoma in the Marshall rat. The gradual growth soon reaches its peak and in a short period of time the tumor recedes and disappears completely. In the parabiotic Marshall rats, in practically all cases where the tumor does develop, it fails to grow as well as in the controls and recedes much more rapidly. In the first two instances on chart 2 it should be noted that the rapid growth took place only after the August animal had died. The obvious conclusions are (1) that the susceptible animal does not transmit any of its characteristics to the immune one; (2) that parabiosis reduces the rate, intensity, and duration of growth of the Jensen rat sarcoma in the Marshall rat. This is probably due to the ill health of the animal in parabiosis.

In a second series, tests were made to determine whether protective substances are transmitted from the immune to the susceptible animal, in other words, whether passive immunity can be developed in this manner. The susceptible Marshall rats were inoculated with Flexner rat carcinoma (FRC). Nine pairs were operated upon; six pairs survived the four day post-operative period and were inoculated with the tumor. Two survived for a period of over two weeks, which allowed chartings



Charts 1 and 2

enough to make a fair comparison with the control. Chart 3 shows that the growths in time of appearance, and rapidity of development are only slightly behind those of the controls.



Charts 3 and 4

CONCLUSIONS

From these experiments it is quite logical to conclude that parabiosis does not increase the susceptibility of the immune animal nor does it give immunity to the susceptible one, and that whatever the substances are that make for growth in the susceptible and for non-growth in the immune animal, these properties are not carried over by the parabiotic union. The lesser size of the tumor and the lesser rapidity of its growth in the susceptible partner can be explained by the setback that the operation has produced and by the interference with the normal activities of the animal, and it is unnecessary to assume as do Albrecht and Hecht that this phenomenon is one of partial artificial immunity similar to that produced by the injection of homologous tissue, since such immunity is known not to affect tumors after growth is begun but only to prevent inoculation. While the number of animals employed in these experiments is rather small, the results, taken in conjunction with those of other observers in this field, are sufficiently consistent to make it unnecessary to repeat them in a larger series.

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THE PROTEIN CONTENT OF THE WHOLE BLOOD AND PLASMA IN CANCER

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In 1919, Dr. William S. Stone and the writer (1) reported a study of the chemical composition of the blood in cancer cases, describing the results of determinations of the sugar and the non-protein nitrogen constituents of the blood. It has been a question for years whether the proteins of the blood are increased or decreased in cancer. Only recently Robin (2) stated that the serum protein is increased, but, like so many other investigators, he based his conclusions on the study of only a few bloods. Therefore, it seemed advisable to investigate a sufficient number of cases and compare the results with those obtained in other diseases.

The nitrogen of the whole blood, serum, or plasma was determined by the Kjeldahl method, and the hemoglobin with Dare's hemoglobinometer. Cancer bloods were obtained from the wards of the Memorial Hospital, and the others from the Roosevelt Hospital.

Forty-three bloods were examined. Tables 1 and 2 show the results obtained on cancer bloods, the only difference being that table 1 refers to serum and table 2 to plasma. Table 3 contains the results on bloods from other pathological conditions.

The protein content of the whole blood depends to such a large extent upon the amount of hemoglobin present that we should expect a large variation. Thus, in table 1 whole blood protein varies from 12 to 19 per cent, with an average of 16.4 per cent.

TABLE 1

Protein of whole blood and serum in cancer

NUMBER	DIAGNOSIS	WHOLE BLOOD PROTEIN*	SERUM PROTEIN	DARE HEMOGLOBIN
		per cent	*per cent*	*per cent*
1	Papilloma of bladder	12.1	7.7	40
2	Carcinoma of bladder	17.6	6.6	80
3	Carcinoma of bladder	16.2	6.0	90
4	Carcinoma of prostate	19.0	7.3	
5	Carcinoma of prostate	19.1	6.7	80
6	Carcinoma of prostate	12.1	6.9	55
7	Carcinoma of prostate	16.2	8·2	
8	Carcinoma of parotid gland	17.8	6.9	
9	Carcinoma of uterus	14.1	7.1	51
10	Carcinoma of rectum	18.1	6.4	90
11	Epithelioma of jaw	16.2	6.0	85
12	Epithelioma of tonsil	16.8	6.6	75
13	Neurosarcoma of arm	18.2	7.7	75

*Nitrogen × 6.25 = protein.

TABLE 2

Protein of whole blood and plasma in cancer

NUMBER	DIAGNOSIS	WHOLE BLOOD PROTEIN*	PLASMA PROTEIN	DARE HEMOGLOBIN
		per cent	*per cent*	*per cent*
14	Carcinoma of bladder	16.9	7.1	75
15	Carcinoma of bladder	13.2	5.3	60
16	Carcinoma of bladder	16.1	5.6	
17	Carcinoma of bladder	18.8	7.1	
18	Carcinoma of uterus	15.5	5.6	60
19	Carcinoma of breast	14.7	6.1	75
20	Carcinoma of breast	15.7	5.9	
21	Carcinoma of rectum	14.4	8.1	
22	Carcinoma of rectum	15.0	5.9	
23	Carcinoma of liver	15.8	6.1	
24	Carcinoma of larynx	14.7	6.7	
25	Carcinoma of stomach	8.7	5.1	
26	Teratoma of testis	18.2	6.7	
27	Keloid of chest	19.3	7.4	

*Nitrogen × 6.25 = protein.

In table 2 the protein varies from 13 to 19 per cent with an average of 16.3 per cent, if we except case no. 25, in which the blood was very anemic with a protein value of only 8.7 per cent.

In table 3 the protein varies from 12 to 20 per cent with an average of 17.6 per cent. Hemoglobin readings were, unfortunately, not done in these cases but they would undoubtedly have averaged higher than in the cancer cases and would account for the slightly higher protein value of the whole blood.

If there is an increase or decrease in the amount of protein in cancer blood, it would show in the serum or plasma. Here we can see that the variation is very slight. Table 1 varies from 5.9 to 8.1 per cent with an average of 6.8 per cent; the patient whose blood had the highest value died the day after the blood was taken. The plasma protein values of table 2 varied from 5 to 8.1 per cent, with an average of 6.3 per cent. The plasma values of other pathological conditions varied from 5.3 to 8 per cent, with an average of 7.0 per cent.

TABLE 3

Protein of whole blood and plasma in other diseases

NUMBER	DIAGNOSIS	WHOLE BLOOD PROTEIN*	PLASMA PROTEIN
		per cent	*per cent*
1	Hematoma of kidney	19.8	7.2
2	Tuberculous glands	16.4	5.4
3	Colon bacillus infection	18.2	5.8
4	Paralysis agitans	19.6	7.9
5	Pleurisy	15.3	6.0
6	Ear infection	19.6	8.0
7	Retroversion of uterus	16.0	6.9
8	Urticaria	17.9	8.1
9	Possible adhesions	19.6	7.6
10	Neurasthenia	17.8	7.2
11	Neurasthenia	17.3	7.4
12	Pyelitis	18.4	8.0
13	Pyelitis	12.3	7.9
14	Pyelitis	20.0	5.9
15	Pyelitis	18.4	7.2
16	Pyelitis	16.3	8.0

*Nitrogen × 6.25 = protein.

Loebner (3) reported results on 44 cases of cancer. She employed the refractometric method, using serum obtained from finger blood. Her figures varied from 4.2 per cent to 9 per cent with an average of 7.3 per cent, which corresponds very closely with the average of this series. She considered 8.2 per cent the average for normals since that is the average which Heudorfer obtained in a study of five cases. This would indicate that the protein of the serum is somewhat reduced in cancer. If, however, we compare the average in cancer cases with the average in cases of other pathological conditions, we can see that there really is no difference in the amount of protein present.

CONCLUSION

Proteins of the blood plasma are neither decreased nor increased in cancer cases as compared with other hospital patients.

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PROBLEMS IN CANCER RESEARCH[1]

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It is a pleasure to announce that a Department for Cancer Research has again been established in St. Louis by the Barnard Free Skin and Cancer Hospital. The material available is the laboratories and what can be developed from the more general biological side within them, and the clinical material.

The plan which is being used for the study of cancer divides the work into several distinct groups. One is a general study of the properties of the various cells of the body and the peculiarities of the mechanisms of growth, division, differentiation, and function of these cells. A second is a close study of the clinical course of the disease and the results of methods of treatment and diagnosis. A third is an attempted analysis of isolated facts now fully demonstrated, and their immediate relation to clinical and pathological data at hand.

For the immediate time, one problem which seems to be most pressing is the improvement of methods of diagnosis, especially the diagnosis of internal cancer. Early diagnosis of cancer of the breast has prolonged the life of many patients for at least ten or twenty years. The same is true for other superficial growths. Barring a few exceptional cases, methods for the diagnosis of internal cancer have not reached the same degree of perfection, as, for instance, cancers of the intestinal canal. If by chance a cancer of the intestine causes early obstruction, a diagnosis may be made and the treatment may be hopeful. How few cancers of this region give, however, this early symptom!

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[1] Presented before the American Association for Cancer Research, Cleveland, Ohio, March 24, 1921.

Another of the immediate problems is the betterment of methods of treatment now available. The third and most important of all is the etiology of this disease. It is most important, because through its elucidation and even in its study all other problems must become simplified. Through it alone, barring some peculiar accident, can the hopes for ultimate success in treatment come. The greatest effort in cancer must be exerted, therefore, in this direction.

What is known about cancer today, aside from clinical considerations, is little more than was known many years ago. This is appalling but true, and the question arises: Is it not due to the fact that the general notions of the cell, its structure and properties have not materially advanced during this time? The problem of the etiology of cancer when resolved into terms of the cell may be quoted thus: Is the cancer cell a cell different from normal cells, or is it a normal cell suffering a continuous external growth stimulus? Since the application of the theories of cellular growth now in vogue have failed to answer this question, there remain open but two channels of attack; the hit and miss method, and a further probing into the nature of the mechanisms of cellular growth, division, differentiation, and function.

Cancer, no matter how it is taken, represents a break in the normal growth-regulating conditions of the body. Without a knowledge of the normal growth-regulating mechanism, it is to be expected that the etiology of cancer will remain obscure, unless it is proved that the disease is the result of some definite foreign agent or stimulus—parasite, or otherwise—which may be isolated.

It is known that the body is composed of cells. The minute metabolism of these cells, the mechanism of their formation, even the simplest constituents of their life are, however, based only on theories. The leucocytes are compared to the amoeba. One talks glibly of cellular metabolism, when he probably means body metabolism. Is this so-called cellular metabolism the work of the cell or is it the work of the organ or the body as a whole? In beginning a few years ago the study of the cells in tissue culture, it was of interest to note that the leucocytes, both the various mononuclear as well as the polymorphonuclear types, show no ability to grow and divide like other cells. Their movements are passive reactions to external conditions. A connective tissue cell or a heart muscle cell suffering from the effects of certain toxic poisons can be made to resemble the small and large mononuclear cells of the inflammatory exudate. These latter cells have lost, also, all ability for further growth under the conditions in which they had previously grown or under any other conditions it has been possible to impose upon them. In actively growing culture many of these fixed tissue cells, unaffected by foreign poisons may also show such changes after they have been preyed upon by actively growing cells.

The polymorphonuclear leucocyte is a cell differentiated in a special locality. Lymphocytes undoubtedly are found in the same locality as well as in lymph-nodes. Whether the large number of mononuclear cells of the chronic inflammatory area or about cancers have this origin is still a very much open question. In fact, the work of Maximow has not been confirmed. (See Stockard (1).) That these cells contain enzymes and bacteriolytic substances which are liberated when they break down is, again, well established. What is not known are the conditions under which these cells live in the areas about a cancer and whether they do liberate these substances under ordinary conditions there. It seems evident, therefore, that they might as well be considered the result of active growth or the presence of toxic substances, as to be thought of as antagonists to these conditions. In chronic inflammations, it is generally where the bacteria are most active that they are most prevalent. In the tissue culture it is the actively growing tissue cells that show the true antagonistic action to growing bacteria. The lymphocytic cells and the rounded-off connective tissue cells intermingle in the cultures with the bacteria. The connective tissue cells, the heart muscle cells, and other actively growing cells cease to grow when they come within the zone of bacterial action. The bacteria stand them off as they also stand off the bacteria (2).

This absence of an absolute knowledge of many of the most important functions of body cells must in itself make it very unsafe, therefore, to draw any far reaching functional conclusions from morphological data. It makes it absolutely necessary to draw one's conclusions from the clinical facts alone. This applies not alone to treatment, but also to other experimental results. The laboratory in Saint Louis is well situated in this regard in that it is a definite part of the clinic, and also allows the broader type of biological research which in the light of present knowledge must form an intimate part of the cancer laboratory.

PROBLEMS UNDER CONSIDERATION

1. Studies on oxidation in actively growing and differentiated cells, especially in reference to their growth

In a previous communication (3) before this society evidence was given by the author to show that connective tissue cells and simple mesenchymatous and embryonic muscle cells show no evidence of an internal organization suitable for growth. They act more like homogeneous fluid systems, leaving aside the nucleus and the astral center. Growth in them can be explained entirely as a surface tension phenomenon, the energy of the process being effected by the formation of a substance within the cell, which is insoluble in circulating body fluid, but soluble in dead cells, fibrin, and products of disintegrating cells. A cell forming this substance and brought in contact with fibrin suffers a decrease in surface tension along the line of contact. The further details of the organization peculiar to growth is determined by these contacts. The order of the contacts of a growing and dividing heart muscle cell is different from a rhythmically contracting one.

For a long time it has been known that actively growing tissue cells, such as those taken from young embryos and sarcomata commence their growth in the culture within one or two hours or much earlier than adult tissues. This is seen in a study of tissues of embryo chicks. The cells from a fragment of the heart of a two, three, or four days old chick embryo will commence to grow almost at once when placed in the culture. About fragments of a ten day old embryo heart such activity is not observed until after six or twelve hours, and about fragments of a fifteen day old chick embryo heart after fifteen or twenty hours; while about young adult heart fragments this latent period is often as long as twenty-four or forty hours.

In a previous publication (4), the writer reported a study of the relation of oxygen to these changes in the cell. A method was developed which allowed accurate regulation of the amount of this gas in the chamber about the cells. The tissues especially studied were fragments of the heart and body wall of fourteen, fifteen, and sixteen days old chick embryos. It was found that the growth was as active in an atmosphere of 9 per cent oxygen as in pure oxygen, and ceased in atmospheres which contained slightly less than 6 per cent of this gas. It became of interest this year to see whether the more actively growing tissue might not show variations in these figures. The question that arose was whether the less actively growing tissues did not have to form or in some other way liberate the substance or substances which actively change surface tension or produce energy. The short latent period in the more actively growing cells suggested a supercharging of this substance or substances.

In 1903, Fletcher (5) showed that a muscle of the adult frog will give maximum contractions every five minutes for a period of two hours in an atmosphere of pure nitrogen. In the work on organization of isolated rhythmical, contracting heart muscle cells in the tissue culture, the author has found evidence to show that the chemical changes of muscular contraction are similar in certain stages to those of growth. If muscle will contract for a time without oxygen the question that arises is: Will not cells fully differentiated for growth show the same reaction (6)?

The tissue so far tested has been cells from the hearts of chick embryos of various ages, ranging from four to fifteen days. Fletcher's experiments were repeated with contracting hearts and heart muscle fragments of these embryos isolated in the tissue culture. These cells will contract for from twenty to twenty-two hours in an atmosphere of nitrogen. In the same oxygen-free atmosphere, the cells from fragments of four and five days old chick embryos will grow from four to six hours. This growth commences, however, always after a considerably longer latent period than that in an atmosphere containing oxygen. It is active for this short time, after which the cells invariably disintegrate rapidly. The period of growth in these hanging-drop cultures is very much longer in air; the early disintegration is rarely or never seen; and when it occurs, it is always later. To obtain a similar growth about the fragments of the heart of ten days old chick embryos 1.8 per cent oxygen was required and about fragments of fifteen days old chick embryos 4.5 per cent.

These experiments have not only been applicable to the problem in question, but have given a method of differentiating functionally the actively growing young embryonic cells from those showing greater degrees of differentiation. In the case of these cells they have also shown the necessity of oxygen for the maintenance of structure. Whether this method may be used for differentiating any actively growing tissue is a problem yet to be considered.

STUDIES OF PLANT CANCERS

Another method which has appealed to us in attacking the cancer problem has been the study of plant cancers. These represent an active proliferative growth, the etiology of which is known. Chambers (7) has undertaken this problem. He was interested during last summer in investigating the metabolic activity of the organism, Bacterium tumefaciens, in culture, and in the course of this study noted that these organisms produce alkali in the culture even in the presence of sugar, which they also cause to disappear rapidly. Smith, by a different method, had noted that ammonia is formed in the cultures of these organisms. Harvey (8) more recently, also, noted that the tissue of these tumors is more alkaline than normal plant tissue. The work of Chambers would indicate, therefore, that the increase in the OH-ion in the tissue of these tumors is due to the etiological agent, which produces alkali in the cultures at least. Chambers is now attempting to see if under the condition imposed on them by the plant, they also form it.

These facts attracted our attention to the work of Menten (9), by which an alkalosis was demonstrated in the blood of cancer patients; and this question is now under investigation. The various methods of testing were critically reviewed and the indicator method was selected. The tests are made on a dialysate of venous blood, which is taken under oil and kept unexposed to air throughout the whole procedure. The results so far obtained have been interesting. In the early part of the disease, these patients show no evidence of change in the reaction of their blood when it is taken from a region far removed from the cancer. A definite alkalosis is observed only when the cancer has grown to considerable extent and involves neighboring glands and tissues. The blood of a patient with a beginning carcinoma in the lip is normal. A definite H-ion change in the blood is not seen until the glands of the neck are involved, and then increases gradually as the disease progresses. The same is true for carcinoma of the intestine. A small annular carcinoma of the intestine with no or but little glandular involvement may show by the indicator method no change in the alkalinity of the blood. Sharp changes are always seen, however, when liver nodules are present. The CO_2 relations in the blood of these patients is now being studied. It is possible that by this means much concerning the significance of this alkalosis may be determined. The anemia may also have an effect.

The importance of these experiments is still to be ascertained. That the alkali is probably produced in the malignant tumor is indicated, however, in the study of one patient suffering from a sarcoma of the forearm. Blood taken directly from a vein leading from the tumor showed a pH of 7.5, while in the other arm the blood pH was 7.3, or normal. This case illustrates the difficulty of using such a method for a clinical test. The body compensates more readily for acid than alkali, but at the same time there is a rapid elimination of alkali by the kidney. In the case of acid, the compensating mechanism gives evidence. To what extent this can be used for determining the presence of alkali is yet to be determined. In the case cited, there was evidently alkali being given into the blood by the tumor, but it was not detectable in the other arm. There was no glandular involvement in this case.

What the further studies of this reaction will open up and the further studies of the bacteria of the plant tumors may reveal is a question which the future alone can answer. It seems, however, that this method may yield something important.

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THE INFLUENCE OF HEREDITY IN DETERMINING TUMOR METASTASES[1]

STUDIES IN THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE

Sixteenth Report

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Tumor metastases in this stock are somewhat rare. In 29,000 necropsies, furnishing something over 4000 primary spontaneous tumors, mostly malignant, only about 19 per cent of the tumors have metastasized. It has been the general opinion of pathologists that the formation of metastases is a distinguishing property of malignant tumors. The infiltration of tumor cells into tissues adjacent to the neoplasm, and the entrance of these cells into the lymph and the blood streams are the usual mechanical bases for secondary growths. But all these conditions exist in innumerable instances, both in human and in animal tumors, without the occurrence of secondary growths. For example, females 7821 and 9260 of this stock (see chart 11, strain 508, p. 158) each had a large and highly malignant infiltrative mammary gland carcinoma of relatively long standing. In both instances there were multiple tumor emboli in the lungs, but no secondary growths arose from these emboli,—i.e., the mechanical basis was there in each case, the occurrence of tumor did not follow.

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[1] Presented before the American Association for Cancer Research, Cleveland, Ohio, March 24, 1921.

Ewing (1), in his chapter on metastasis, speaks of "a peculiar susceptibility of some tissues to develop secondary tumors," but later states that "the mechanism of circulation will doubtless explain most of these peculiarities, for as yet there is no evidence that any one parenchymatous organ is more adapted than others to the growth of embolic tumor cells." There would seem, then, to be some factor, other than the mechanical one, which determines the incidence and location of metastasis, even though it be minimized, as Ewing has done in the paragraph cited.

Again, Ewing states that in highly vascular tissues like the lip, stomach, and testicle, very small carcinoma may yield distant metastases. But these very organs are among those whose tumors rarely metastasize in this stock. For example, among the lip carcinomas in this stock none has metastasized; among the testicle tumors (2), one metastasized locally, while five out of six stomach carcinomas (3), (4), showed metastases in regional lymph-nodes only, but no vascular metastases in the liver or elsewhere. And this in spite of the close resemblance of these tumors to human tumors of similar type in similar organs.

In this stock, mammary gland carcinoma and sarcoma rarely metastasize into regional lymph-nodes, in marked contrast to human breast tumors of the same type. Indeed, in this stock, mammary gland tumors can be seen growing up to the lymph-node but not invading it, even by extension. On the other hand, pulmonary metastases from mammary gland carcinoma and sarcoma are common in this stock, again in contradistinction to the reported behavior of human breast tumors of the same types.

These divergencies (which are typical of the many divergencies) in the metastasis behavior of tumors of similar types and in similar organs, require some explanation other than merely the mechanical tendency of certain types of tumors to metastasize in certain locations, and that there is some other factor is generally recognized by pathologists, although little progress has as yet been made in demonstrating what this factor is.

The matter of tumor metastasis in this laboratory has been under observation for twelve years, and the data here given are based on over 29,000 necropsies, among which every instance of metastasis is known. The charts used to demonstrate the points made in this report are perfectly typical. The non-tumor fraternities (where there are any) have been omitted, in order to save space and to show exactly where these tumors occurred.

It must be borne in mind in reading these charts, that, as I have previously stated, tumor metastases in this stock are rather rare, being only about 19 per cent of the primary malignant tumors. A given chart then, which shows many primary tumors, must not be expected to show large numbers of secondary tumors. For example, a chart showing 8 primary tumors, would, if this general average of 19 per cent were carried out, show only 1.5 cases of metastasis, etc. The biological evidence from this laboratory, which explains this low percentage of metastasis from malignant tumors, will be given further on in this report.

From this study three points stand out with striking clearness:

POINT I

In any given strain the metastatic tumors (where there are any) tend to occur in exactly the same organs in which the primary tumors of that strain occur.

Note chart 1, showing part of strain 215 and some of its derivatives. The parent female, no. 3, had a sarcoma-carcinoma of the mammary gland, a malignant adenoma of the liver, and a metastatic sarcoma of the kidney. The parent male, no. 360, came from a strain carrying lung and mediastinal tumors, and he himself was proved heterozygous to tumors in these locations (i.e., he carried them potentially and introduced them in every strain into which he was crossed, although he did not himself show these tumors).

Note here how these particular types and locations of tumors have "segregated out" and certain of them have been transmitted to the branches of the strain here shown, both as primary

PART OF STRAIN 215 AND SOME DERIVATIVES

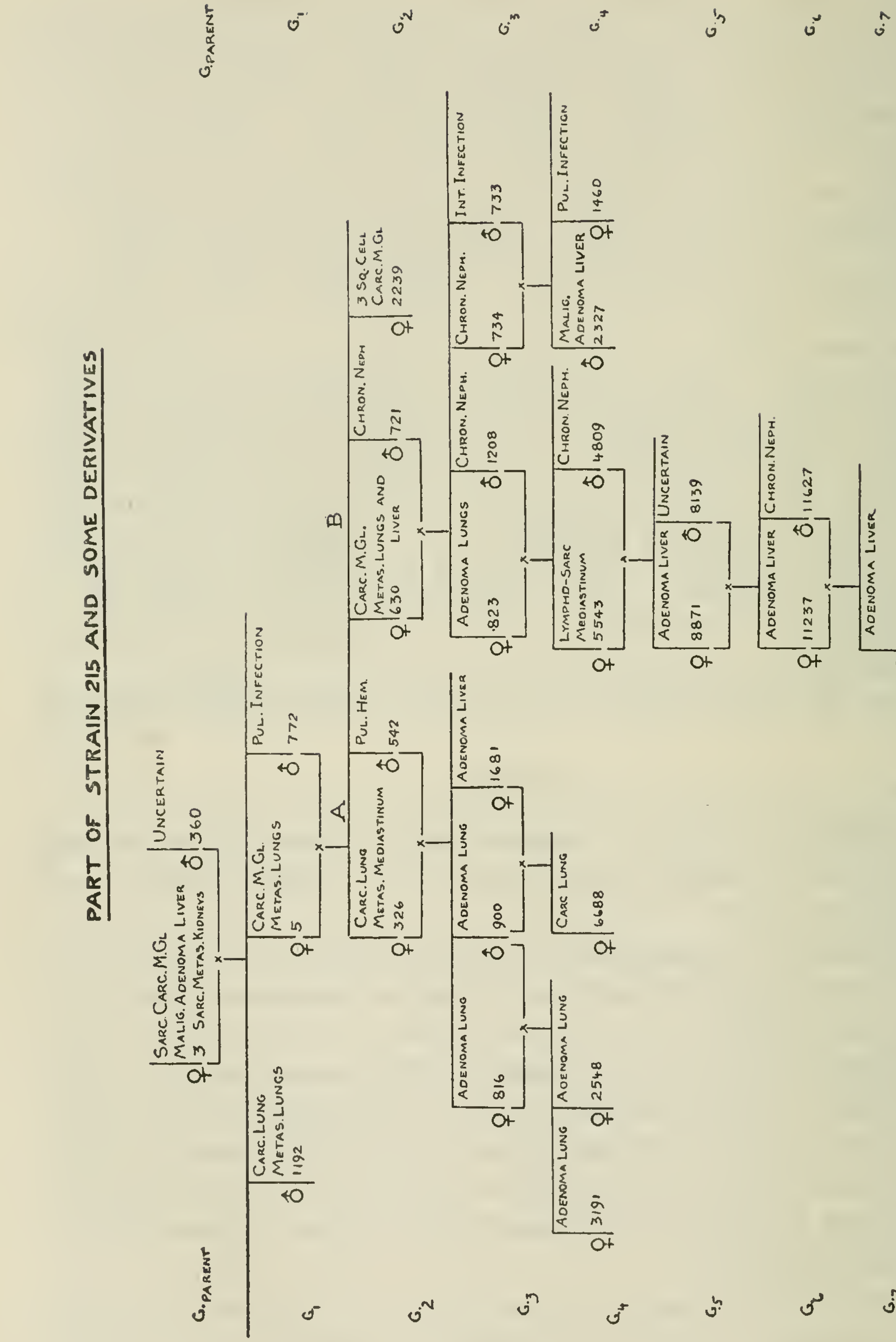


CHART 1

and as secondary neoplasms. In the first hybrid generation, male 1192 had a primary carcinoma of the lung and a secondary carcinoma of the lung. Female 5 had a primary carcinoma of the mammary gland, with multiple secondaries in the lung. Note how the different types and locations of tumors have "segregated out," so that in branch A lung tumors alone have been transmitted, and a 100 per cent lung tumor strain is being extracted. In branch B liver tumors have predominantly been transmitted, from female 630 with a secondary tumor in the liver, so that in one part of the branch a 50 per cent liver tumor strain is being extracted.

In these two branches of the strain, then, female 3 introduced:

a. Primary carcinoma of the mammary gland, in females 5, 2239, and 630.

b. Primary adenoma of the liver, in females 1681, 8871, 11237, and 12207, and in male 2327. (Note the three consecutive generations of adenoma of the liver.)

c. Secondary carcinoma of the liver, in female 630.

d. One case of sarcoma, in female 5543.

Parent male 360 introduced:

a. Primary carcinoma and adenoma of the lung, in males 1192, and 900, and in females 326, 816, 823, 3191, 2548, and 6688.

b. Secondary carcinoma of the lung, in male 1192 and in females 5 and 630.

c. Primary tumor of the mediastinum, in female 5543.

d. Secondary tumor of the mediastinum, in female 326.

Note how female 5543, with a lymphosarcoma of the mediastinum, derived the type of her tumor from her maternal ancestor (female 3) and the location of her tumor from the paternal ancestor, male 360 (who carried these tumors potentially, and transmitted them in every strain into which he was crossed, although he himself did not develop them), just as in the human species, a child might inherit curly red hair from a mother with curly black hair, and a father with straight red hair.

Note how female 5 derived her primary tumor from the maternal ancestor, and her secondary tumor from the paternal ancestor, and how female 630 derived her primary tumor from the parent female, the secondary in the lungs from the parent male, and the secondary tumor of the liver from the parent female—a marked demonstration of the "segregating out" of these things, which is *the heredity test.* Note how, after the second hybrid generation, no further mammary gland tumors occurred in either of these two branches.

Note that there occur no tumors, either primary or secondary, which were not introduced by the parents. Note that the secondary tumors occur in the same organs as the primary tumors.

Chart 2 shows parts of strains 48 and 292. These are two strains derived from the same original mating as was strain 215, that is, female 3 (with a sarcoma-carcinoma of the mammary gland, a malignant adenoma of the liver, and a metastatic sarcoma of the kidney), and male 360 who introduced carcinoma of the lung and the mediastinum. Here again the particular types and locations of tumors introduced by the parents have "segregated out" and been transmitted to the resulting strains, both as primary and as secondary neoplasms.

In the first hybrid generation female 5 derived from the mother a carcinoma of the mammary gland, and from the father a secondary carcinoma of the lung. In the second hybrid generation, female 37 derived from the parent male a carcinoma of the lung, with a secondary carcinoma in the mediastinum.

In the third and fourth hybrid generations, note the outcropping of sarcoma, both primary and secondary, in females 1454, 26, 348 and 396. Liver tumors derived from female 3, also occur in these strains, as shown in females 5743, and 26, and in male 11836. There is also a "pre-cancerous" liver in female 399. Here also lung tumors, derived from male 360, occur primary in females 37 and 399 and in male 11836; and secondary in females 5, 5743, and 396.

Note the occurrence of secondary *carcinoma* of the mediastinum in female 37, of secondary *sarcoma* of the mediastinum in female 396; of secondary *carcinoma* of the lung in female 5, and of secondary *sarcoma* of the lung in females 5743 and 396. Note the three cases of secondary sarcoma in the spleen in females

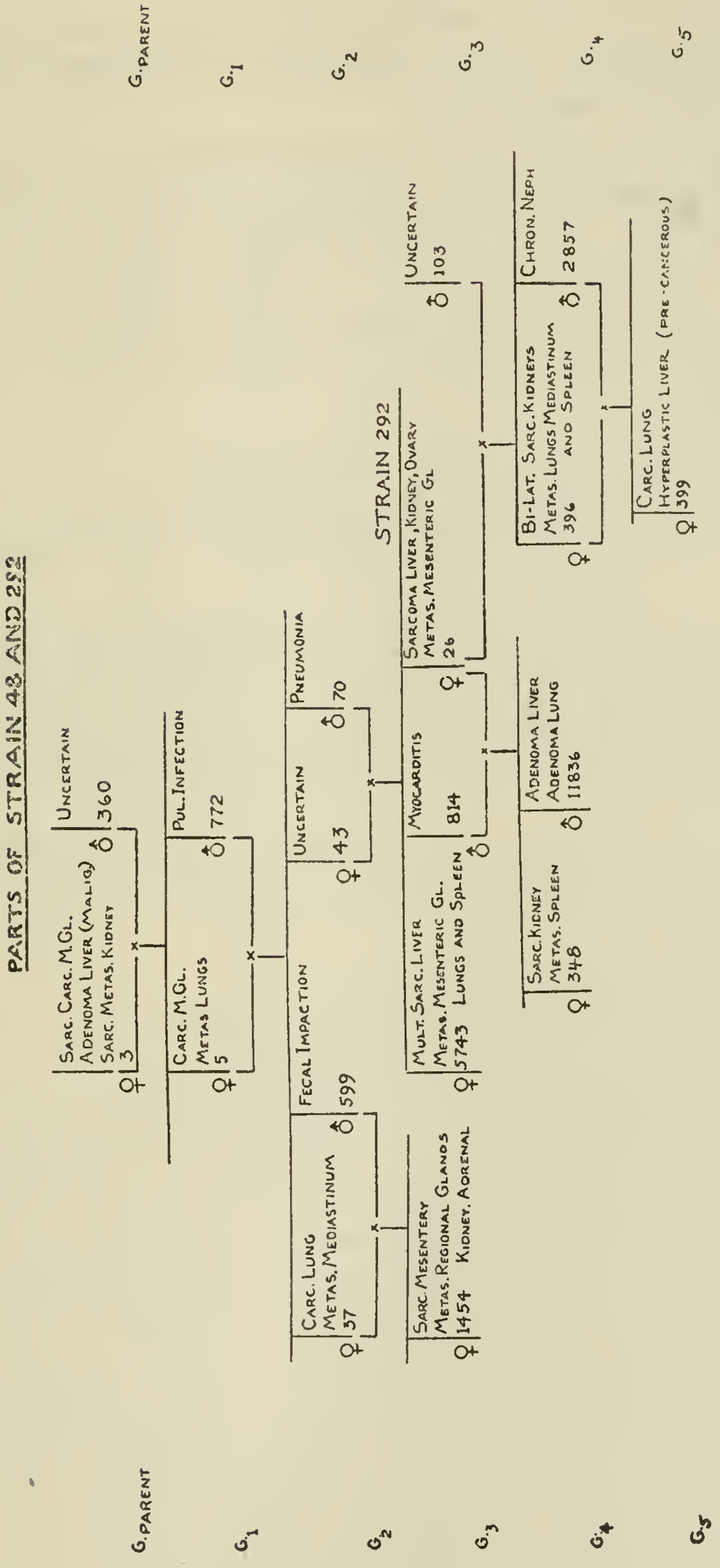

PARTS OF STRAIN 48 AND 292
STRAIN 292
G. PARENT
G.1
G.2
G.3
G.4
G.5
♀ Sarc. Carc. M. Gl. Adenoma Liver (Malig) Sarc. Metas. Kidney 3
♂ Uncertain 360
♀ Carc. M. Gl. Metas Lungs 5
♂ Pul. Infection 772
♀ Fecal Impaction 599
♀ Uncertain 43
♂ Pneumonia 70
♀ Carc. Lung Metas. Mediastinum 57
♂ 599
♀ Sarc. Mesentery Metas. Regional Glands Kidney. Adrenal 1454
♀ Mult. Sarc. Liver Metas. Mesenteric Gl. Lungs and Spleen 5743
♂ Myocarditis 814
♀ Sarcoma Liver, Kidney, Ovary Metas. Mesenteric Gl 26
♂ Uncertain 103
♀ Sarc. Kidney Metas. Spleen 348
♂ Adenoma Liver Adenoma Lung 11836
♀ Bi-Lat. Sarc. Kidneys Metas. Lungs Mediastinum and Spleen 396
♂ Chron. Neph 2857
♀ Carc. Lung Hyperplastic Liver (pre-cancerous) 399
x

CHART 2

5743, 348, and 396. Rarely in this stock, except in strains derived from female 3, have there been any tumors of the spleen either primary or secondary.

Here again, after the first hybrid generation, mammary gland tumors disappear—another indication of how these things "segregate out." The mammary gland tumors preponderated in other strains derived from the same original mating. The dominating tumors, both primary and secondary, in these strains are in the liver, kidney, spleen, mesentery, lungs, and mediastinum.

In these strains, then, female 3 introduced:

a. Primary carcinoma of the mammary gland, in female 5.

b. Primary adenoma of the liver, in female 399 and in male 11836.

c. Primary sarcoma of the liver, in females 5743 and 26.

d. Primary sarcoma of the kidneys, in females 26, 348, and 396.

e. Secondary sarcoma of the kidneys, in female 1454.

f. Secondary sarcoma of the spleen, in females 5743, 348, and 396.

g. Primary sarcoma of the mesentery, in female 1454.

h. Secondary sarcoma of the mesentery, in females 1454, 5743, and 26.

Parent male 360 introduced:

a. Primary lung tumors, malignant and not yet malignant, in females 37 and 399, and in male 11836.

b. Secondary lung tumors, in females 5743 and 396.

c. Secondary carcinoma of the mediastinum, in female 37.

d. Secondary sarcoma of the mediastinum, in female 396.

Note here again, how, though they are much fewer, the secondary tumors arise in the same organs as do the primary.

Chart 3 shows a part of strain 65, branch 2. The parent female, 5738, had three carcinomas of the mammary gland and metastatic tumors in the lungs. She was mated with her brother, male 782, who died of uncertain causes, but who was proved heterozygous to tumors of the mammary gland (he carried them potentially and transmitted them, but did not himself develop them). Note here that with one exception

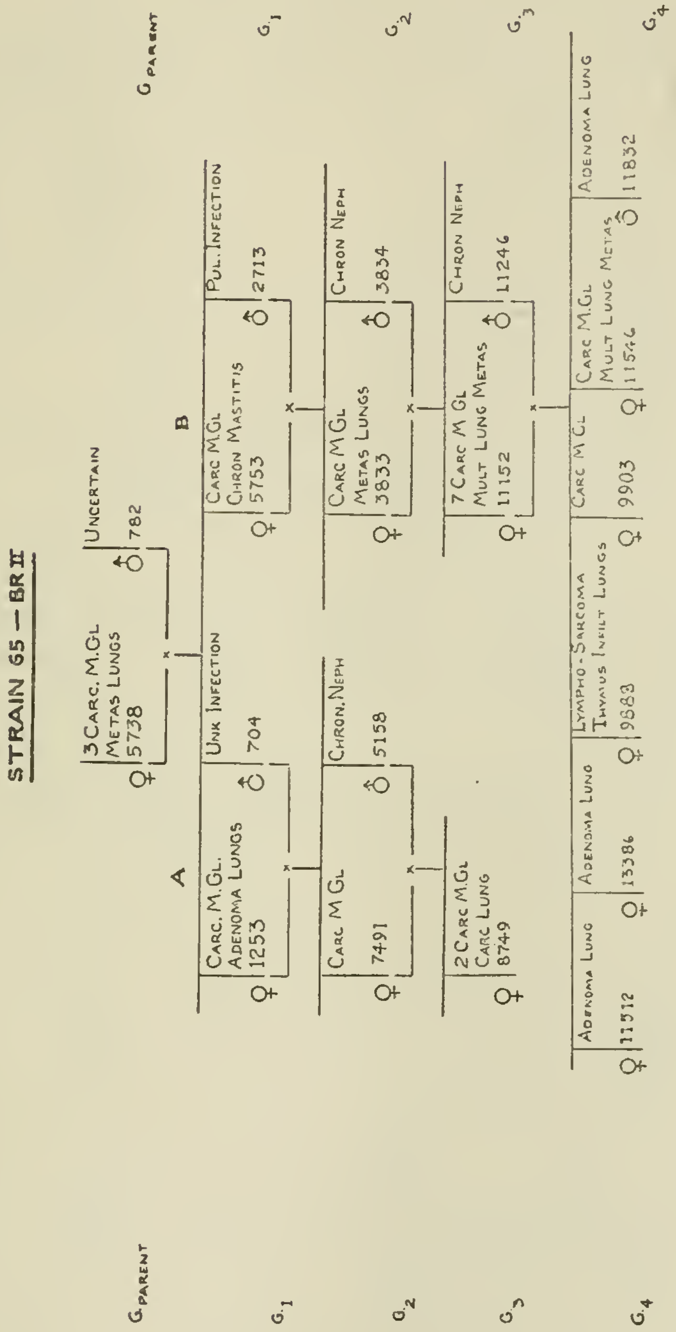

STRAIN 65 — BR II
G PARENT
G 1
G 2
G 3
G 4
A
B
♀ 3 Carc. M.Gl Metas Lungs 5738
♂ Uncertain 782
♀ Carc. M.Gl. Adenoma Lungs 1253
♂ Unk Infection 704
♀ Carc M.Gl Chron Mastitis 5753
♂ Pul. Infection 2713
♀ Carc M Gl 7491
♂ Chron. Neph 5158
♀ Carc M Gl Metas Lungs 3833
♂ Chron Neph 3834
♀ 2 Carc M.Gl Carc Lung 8749
♀ 7 Carc M Gl Mult Lung Metas 11152
♂ Chron Neph 11246
♀ Adenoma Lung 11512
♀ Adenoma Lung 13386
♀ Lympho-Sarcoma Thymus Infilt Lungs 9883
♀ Carc M Gl 9903
♀ Carc M.Gl Mult Lung Metas 11546
♂ Adenoma Lung 11832

CHART 3

(female 9888 who had a lymphosarcoma of the thymus) the only tumors which occurred were carcinoma of the mammary gland and lung tumors, primary and secondary.

Note the five consecutive generations of carcinoma of the mammary gland in branch B, all but females 5753 and 9903 showing lung metastases. Note the heavy percentage of lung tumors primary and secondary in both branches of the strain. Note the negative evidence of the "segregating out" of the different types and locations of tumors, both primary and secondary, in the complete absence from this strain of all tumors of the liver, kidney, mesentery, spleen, pancreas, uterus, etc.

Chart 4 shows a part of strain 338, branch V, A, with partial ancestry. The parent female 3920 had an adenoma of the liver. Note the predominance of primary liver adenoma in this strain, 7 cases (over 43 per cent) and the occurrence of two cases of secondary liver tumor in the same strain, that is, female 8865 with an osteoid sarcoma of the mammary gland, secondary in the liver, and her grandson, male 16370 with an osteosarcoma of the leg, metastasizing in the liver, and also a mesothelioma of the testicle. (Testicle tumors also occur in this strain.)

Chart 5 shows a continuation of the same strain, strain 338, branch V, A (the data being too extensive for a single chart). Chart 5 begins with generation 3 of the family, the mating being between female 8619 and male 8751. Generations 7, 8, and 9 are added to the family in this chart. Note here, also, the further occurrence of liver tumors, there being one additional primary, in female 30469, who showed a primary liver adenoma in addition to seven carcinomas of the mammary gland, a primary lung adenoma, a typical uterine fibroid, and a diffuse endothelial hyperplasia of the glands and spleen. (Needless to say, the mouse was riddled with tumor, little but neoplasm remained.) There were also in this part of the family, two additional cases of secondary liver tumor, *one carcinoma* and *one sarcoma*, that is, female 22263 with three carcinomas of the mammary gland and a sarcoma in the peritoneum near the stomach, with *sarcoma metastases* in the liver, pancreas, and uterine wall, and female 30501 of generation 9, with two carci-

PART OF STRAIN 338 — BR.II A — WITH PART OF ANCESTRY

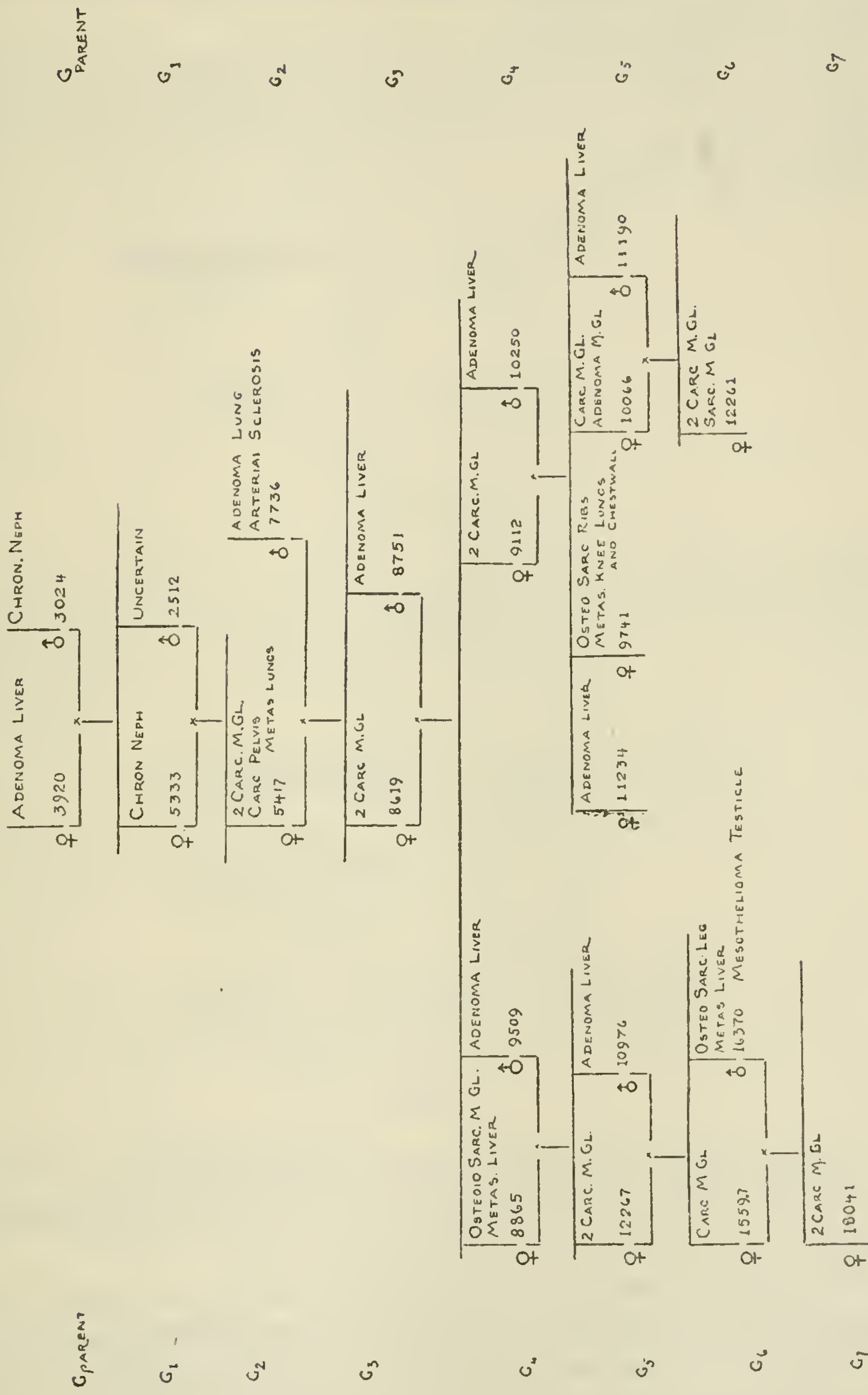


CHART 4

nomas of the mammary gland and the liver absolutely riddled with *carcinoma secondaries.*

It is interesting to note here also the two occurrences of uterine neoplasm, *primary* in female 30469, and *secondary* in female 22263, as this is one of the strains carrying most of the uterine tumors in this stock.

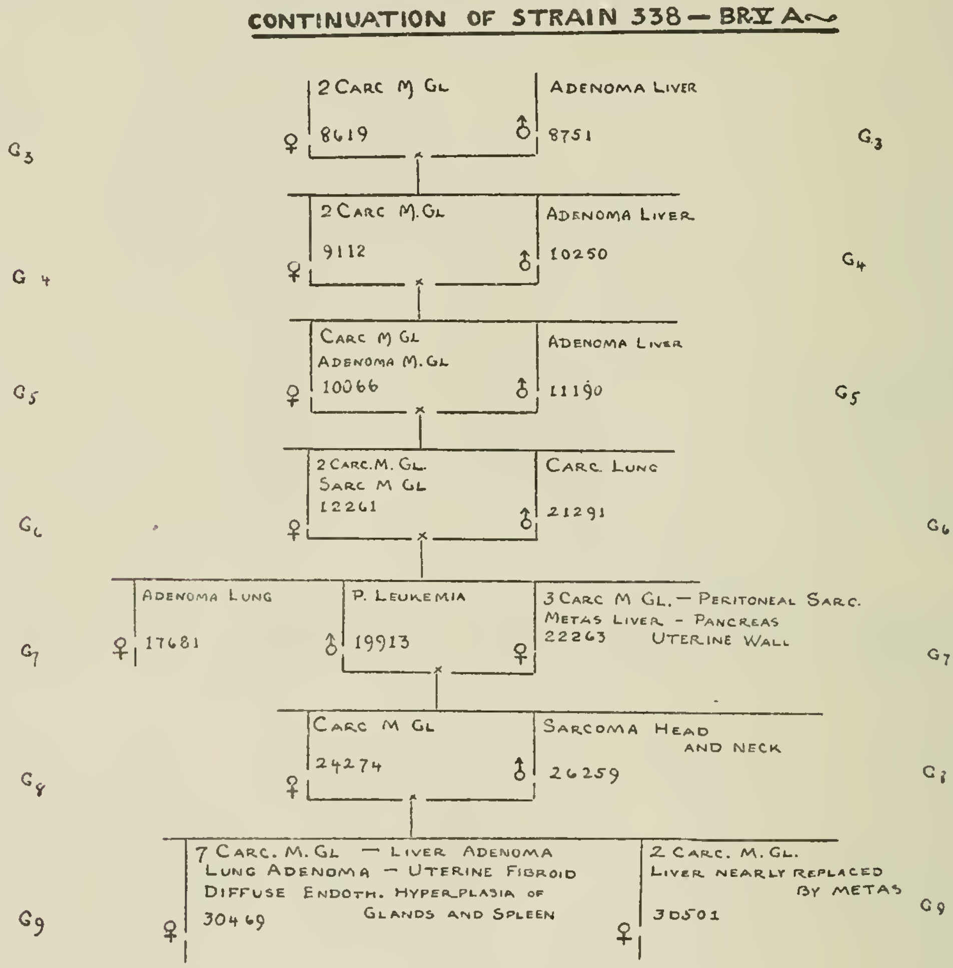


CHART 5

The pancreatic secondary sarcoma also is extremely interesting, as this is the strain which carries the only other pancreatic neoplasms which have occurred in this stock.

The complete ancestry of this strain is shown in charts 6 and 7, chart 6 being the ancestry of parent female 3920, and chart 7 the ancestry of parent male 3024. The prominence of liver tumors, primary and secondary, in the resulting strain is due to the fact that it was bred in from both sides of the family; the

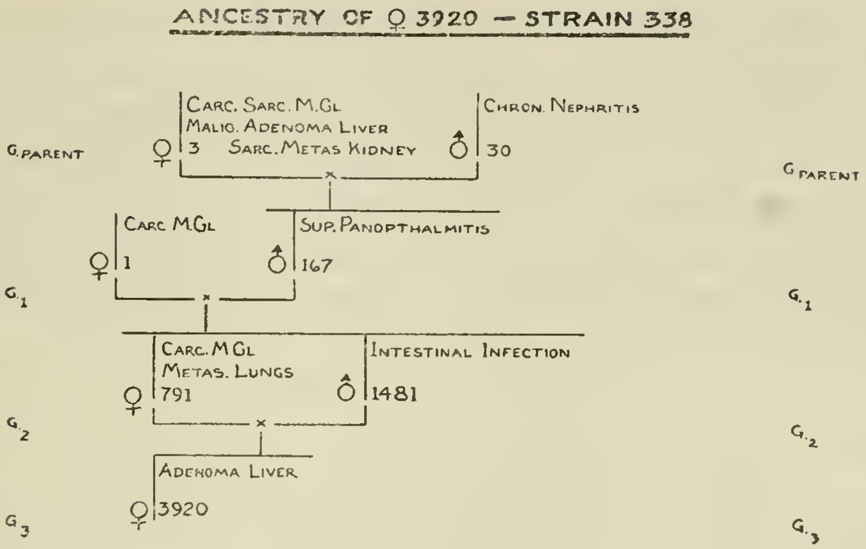


CHART 6

strain thus received "a double dose." Note also the marked outcropping of sarcoma in strain 338, derived from original parent female 3. Sarcoma also was bred in from both sides of the ancestry.

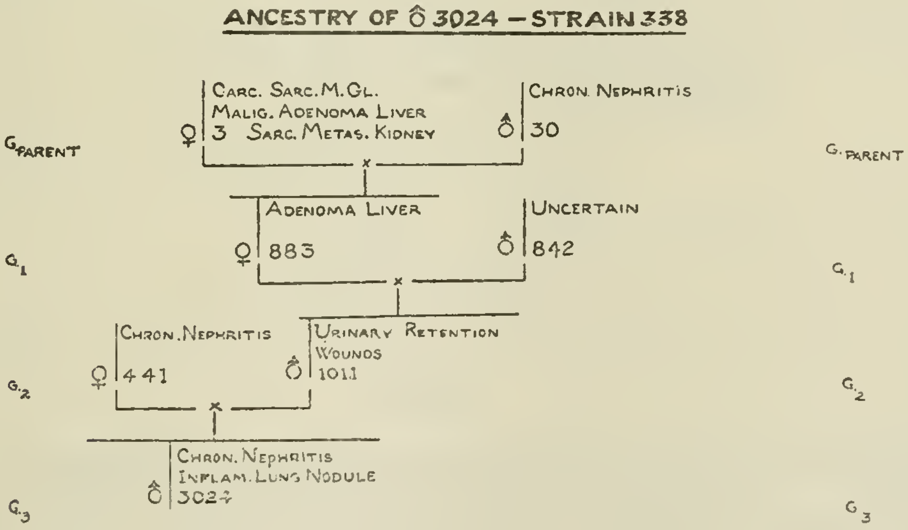


CHART 7

Chart 8 shows a part of strain 465, which was derived from the same original ancestry as strain 338 (ancestry shown in charts 6 and 7). Note here also, the same appearance of liver tumors, two primary and one secondary. This gives us, then, in these two small strains (338, branch V, A and 465) derived from identical original ancestry, 10 primary and 5 secondary liver tumors. Including the ancestry, the number is 13 primary and 5 secondary liver tumors. This is a very high occurrence of liver tumors, both primary and secondary, especially when one considers the fact that outside of this stock there is just one mouse liver tumor on record, a primary tumor of the liver, reported by the Imperial Cancer Research Fund of England. This liver tumor occurrence (in the Slye stock) within a single strain, makes a piece of evidence which is conclusive.

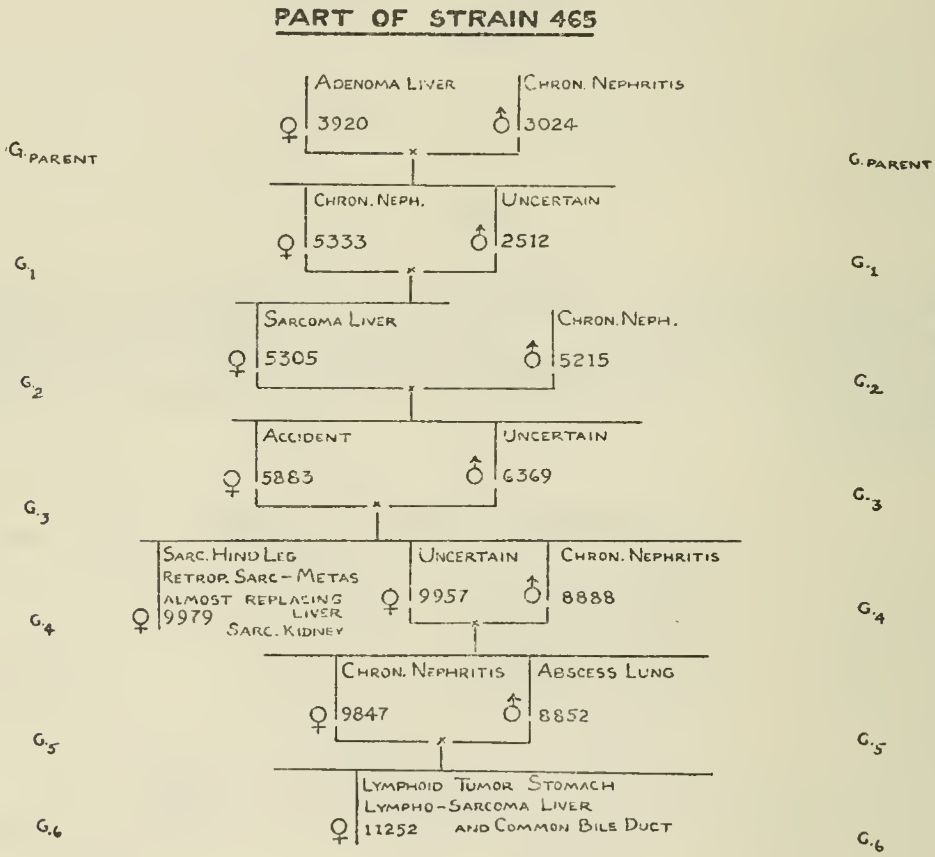


CHART 8

These five strains, represented in charts 1 to 8 inclusive (which, it must be remembered, are perfectly typical) furnish both *positive* and *negative* evidence of the "segregating out" of different types and locations of tumors, and their transmission as such, whether as primary or as secondary neoplasms. They furnish, therefore, both positive and negative evidence that *in any given strain the metastatic tumors* (*where there are any*) *tend to occur in exactly the same organs in which the primary tumors of that strain occur.* Note, therefore, that:

1. Every secondary tumor of the liver in this stock (with the possible exception of two osteosarcomas, which metastasized into nearly every organ in the body, in two mice whose strains have not yet been completely analyzed) has occurred in strains carrying primary liver tumors.
2. Every secondary tumor of the kidney in this stock has occurred in strains carrying primary kidney tumors.
3. Every secondary lung tumor in this stock has occurred in strains carrying primary lung tumors (with the possible exception of the one lung metastasis in strain 164, branch IV, line C).
4. Every secondary tumor of the mediastinum in this stock has occurred in strains yielding primary tumors of the mediastinum.
5. Every secondary tumor of the pancreas in this stock has occurred in the strain which yields primary pancreatic tumors.
6. Every secondary tumor of the spleen in this stock, has occurred in strains carrying primary spleen tumors.
7. Every secondary uterine tumor in this stock has occurred in strains carrying primary uterine tumors.

When this stock, or any other, has been completely analyzed, there is little doubt that it will show that every secondary tumor of any organ whatever has arisen in a member of a strain which has shown, or will show, some cases of primary tumors in that organ (however few they may be). There is little doubt that strain 164, branch IV, line C, of this stock will in time yield a primary lung tumor, although it has not done so yet. There is a very slight inheritance of lung tumor neoplasm tendency in this line of the strain, and it is therefore to be expected that lung tumors of any kind will occur but seldom.

The complete analysis of any strain is a very long and difficult process, and the apparent testimony of the frequent occurrence of secondaries in man in tissues where primary tumors rarely occur is of no help here, as no human strain has ever been even partially analyzed.

Female 3 is listed in these charts as having a sarcoma-carcinoma of the mammary gland. This tumor was unquestionable carcinoma in some parts and apparently sarcoma in other parts. There has been considerable difference of opinion concerning these mixed tumors. Slye, Holmes, and Wells (5) Loeb (6), Lewin (7), Bashford (8), Woglom (9), and some others, diagnose them as such, while Le Count (10) and Ewing (11), etc., consider them to be entirely carcinoma, with pressure distortion of the cells simulating sarcoma in some areas.

The biological evidence of the work in this laboratory unquestionably supports the opinion that there are these mixed tumors. Female 3 with such a tumor, has unquestionably transmitted carcinoma in every strain into which she has been hybridized; and she has equally certainly transmitted sarcoma in some branch of every strain into which she has been crossed (see charts 1, 2, 4, 5 [female 3 shown in ancestry charts 6 and 7], 8 (same parentage charts 6 and 7), Chart 9).

I have preferred the term sarcoma-carcinoma to represent this type of tumor, because according to the biological evidence, it is not a sarcomatous carcinoma, nor a carcinomatous sarcoma, but rather a sarcoma plus a carcinoma, each type of tumor being transmitted separately, as such. Biological evidence, as manifested in heredity behavior, is too fundamental to be ignored.

POINT II

In certain strains there is a tendency for primary tumors to metastasize in certain organs; whereas, in other strains, tumors of the same type, primary in the same organ, even when they are of older and of larger growth, fail to metastasize into those organs.

Chart 9 shows part of strain 73, which was derived from the same female 3, mated this time with male 30. Male 30 came from a strain carrying tumors of the lung, mediastinum, and diaphragm, and was proved heterozygous to tumors of these organs, having been tested in various crosses.

The son of this mating, Jap. W. F. male, died before autopsies were made, and consequently the cause of his death is not known.

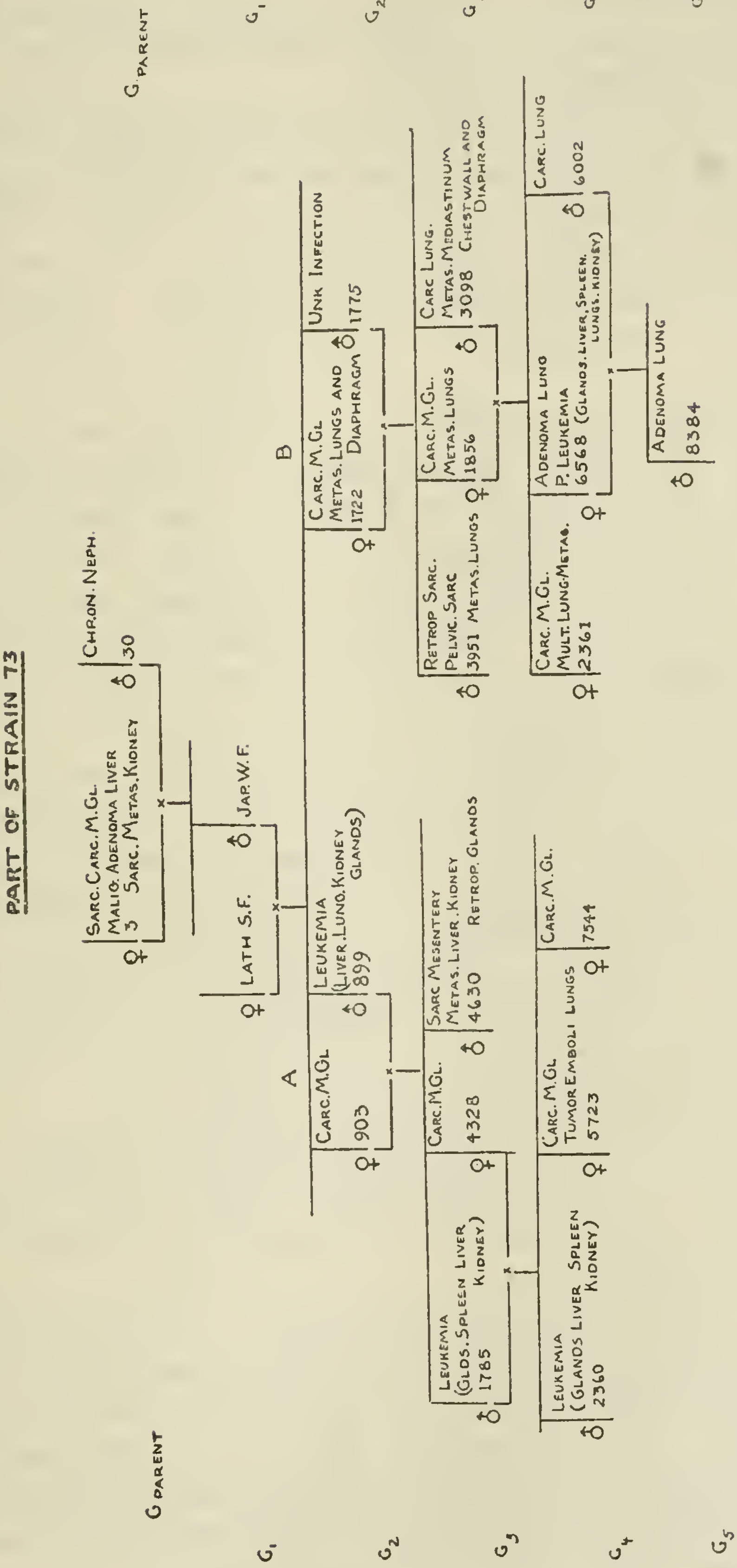

PART OF STRAIN 73
G PARENT
G1
G2
G3
G4
G5
♀ Sarc. Carc. M. Gl. Malig. Adenoma Liver 3 Sarc. Metas. Kidney
♂ Chron. Neph. 30
♀ Lath S.F.
♂ Jap. W.F.
A
B
♀ Carc. M. Gl. 903
♂ Leukemia (Liver, Lung, Kidney Glands) 899
♀ Carc. M. Gl. Metas. Lungs and Diaphragm 1722
♂ Unk Infection 1775
♂ Leukemia (Glds. Spleen Liver Kidney) 1785
♀ Carc. M. Gl. 4328
♂ Sarc Mesentery Metas. Liver, Kidney Retrop. Glands 4630
♂ Retrop Sarc. Pelvic. Sarc 3951 Metas. Lungs
♀ Carc. M. Gl. Metas. Lungs 1856
♂ Carc Lung. Metas. Mediastinum 3098 Chestwall and Diaphragm
♂ Leukemia (Glands Liver Spleen Kidney) 2360
♀ Carc. M. Gl. Tumor Emboli Lungs 5723
♀ Carc. M. Gl. 7544
♀ Carc. M. Gl. Mult. Lung Metas. 2361
♀ Adenoma Lung P. Leukemia 6568 (Glands, Liver, Spleen, Lungs, Kidney)
♂ Carc. Lung 6002
♂ Adenoma Lung 8384

Chart 9

He was crossed with a Lathrop silver-faun female, who also died before autopsies were made. Their offspring, however, are shown in two branches, A and B.

Note how in branch B, every mammary gland carcinoma metastasized into the lungs; even the retroperitoneal and pelvic sarcomas (male 3951) metastasized in the lungs. Note also that with the exception of male 1775 this branch shows 100 per cent of lung tumors, primary and secondary. Note the two generations of metastasis into the diaphragm, female 1722 and male 3098.

On the other hand, in branch A, carcinomas of the same type and of older and larger growth, failed to metastasize into the lungs (females 4328, 5723, and 7544). Note that in female 5723, although the mechanical basis for lung secondaries was present in multiple tumor emboli throughout the lungs, no lung tumor developed. Note that the sarcoma of the mesentery in male 4630 metastasized into the liver, kidney, and retroperitoneal glands, but not into the lungs.

Note the three consecutive generations of leukemia in this strain, in males 899, 1785, and 2360; and the further fact that, although the lungs are one of the principal organs for leukemic infiltration, in males 1785 and 2360 there was no infiltration in the lungs, while in male 899 the lung infiltration was very slight. On the other hand, the marked leukemic infiltration in this family was in the same organs in which the tumors of the parent female occurred, that is, the liver and kidney. The metastatic tumors, also, in this family were in these same organs.

In branch B, however, the 100 per cent lung tumor strain, female 6568 who had pseudoleukemia along with an adenoma of the lung, showed marked infiltration of pseudoleukemia throughout the lungs.

We have here, in these two branches of the same strain, a striking illustration of point II: In branch B all tumors metastasize into the lungs, and pseudoleukemia picks out the lungs predominantly; while in branch A, the tumors of the same type in the same organ and of older and larger growth fail, in every case, to metastasize into the lungs. Even the leukemic infiltration fails to take hold of the lungs in this branch.

Chart 10 shows part of strain 405. In this branch of the family, after the parent generation, although there are five straight generations of mammary gland carcinoma, none of these tumors metastasized into the lungs. Note also the complete absence of primary lung tumors. This branch of the family did not inherit lung tumors, and none occurred, either primary or secondary.

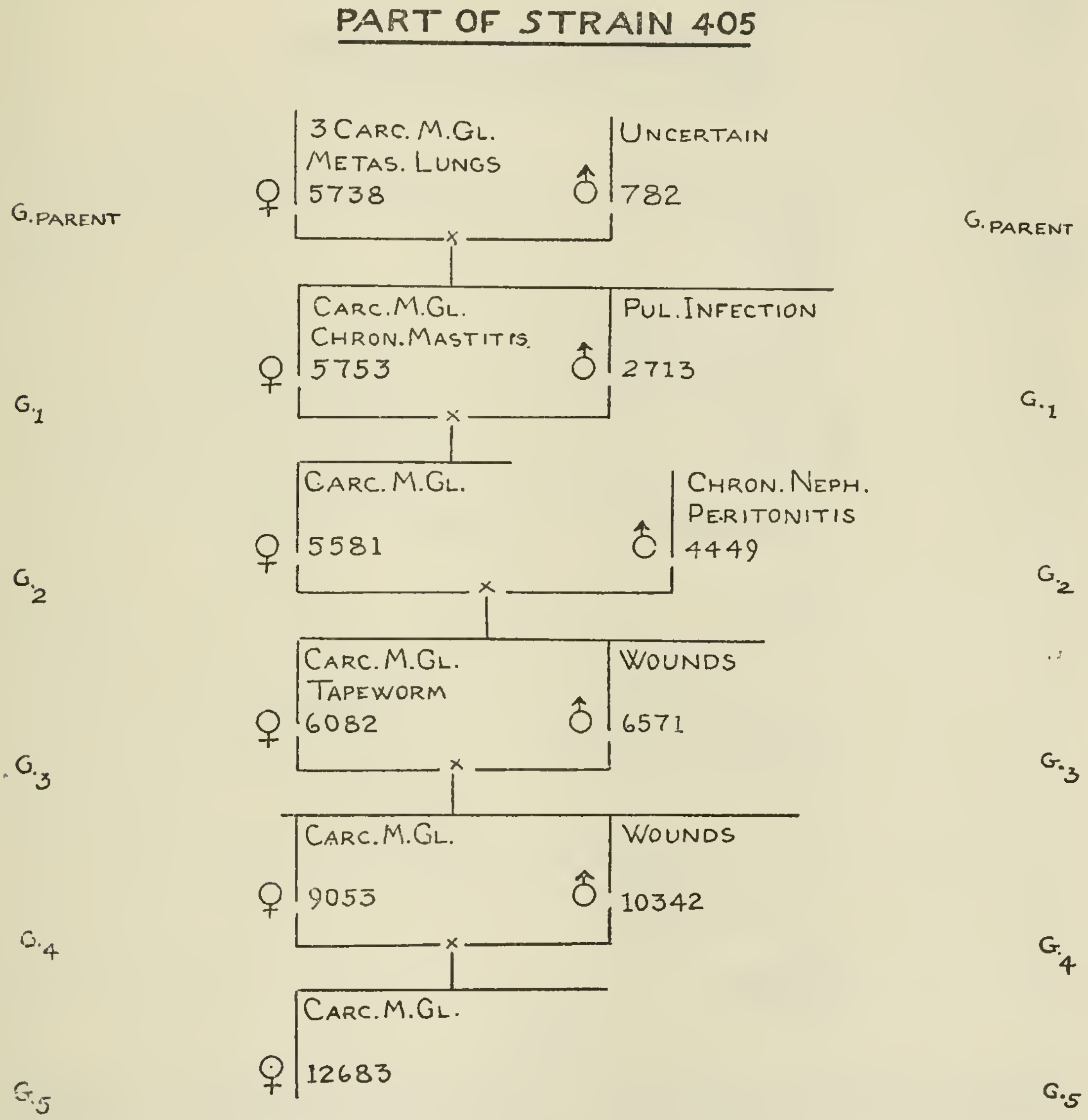


CHART 10

Chart 11 shows one branch of strain 508. Here again through five consecutive generations of mammary gland carcinoma, no lung metastasis occurred. Even where the presence of multiple tumor emboli in the lungs gives certain evidence that cells from the primary tumor were carried to the lungs (in females 7821 and 9260) no lung tumor occurs. The mechanical basis was there; the tumor growth did not follow. This strain also shows no primary lung tumors.

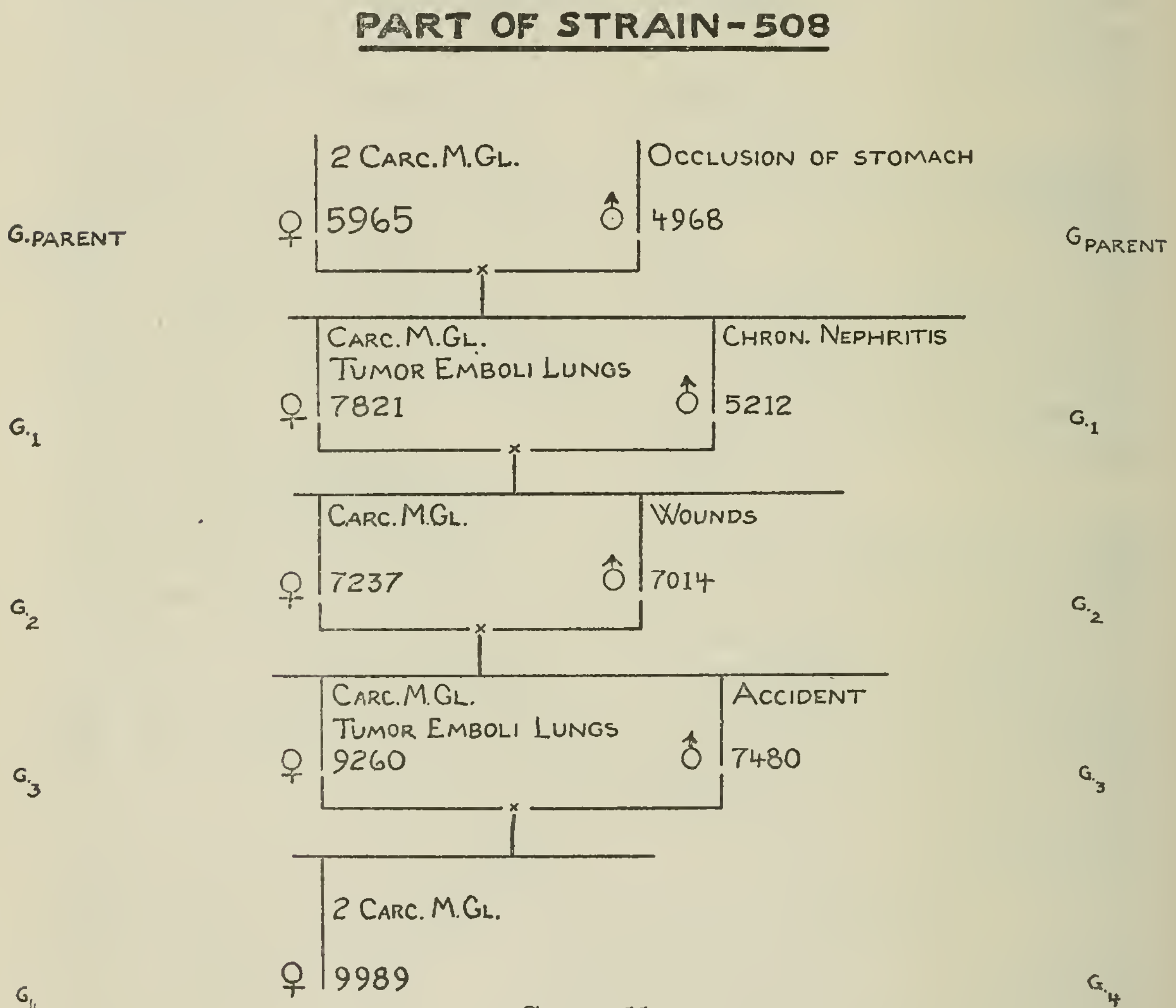
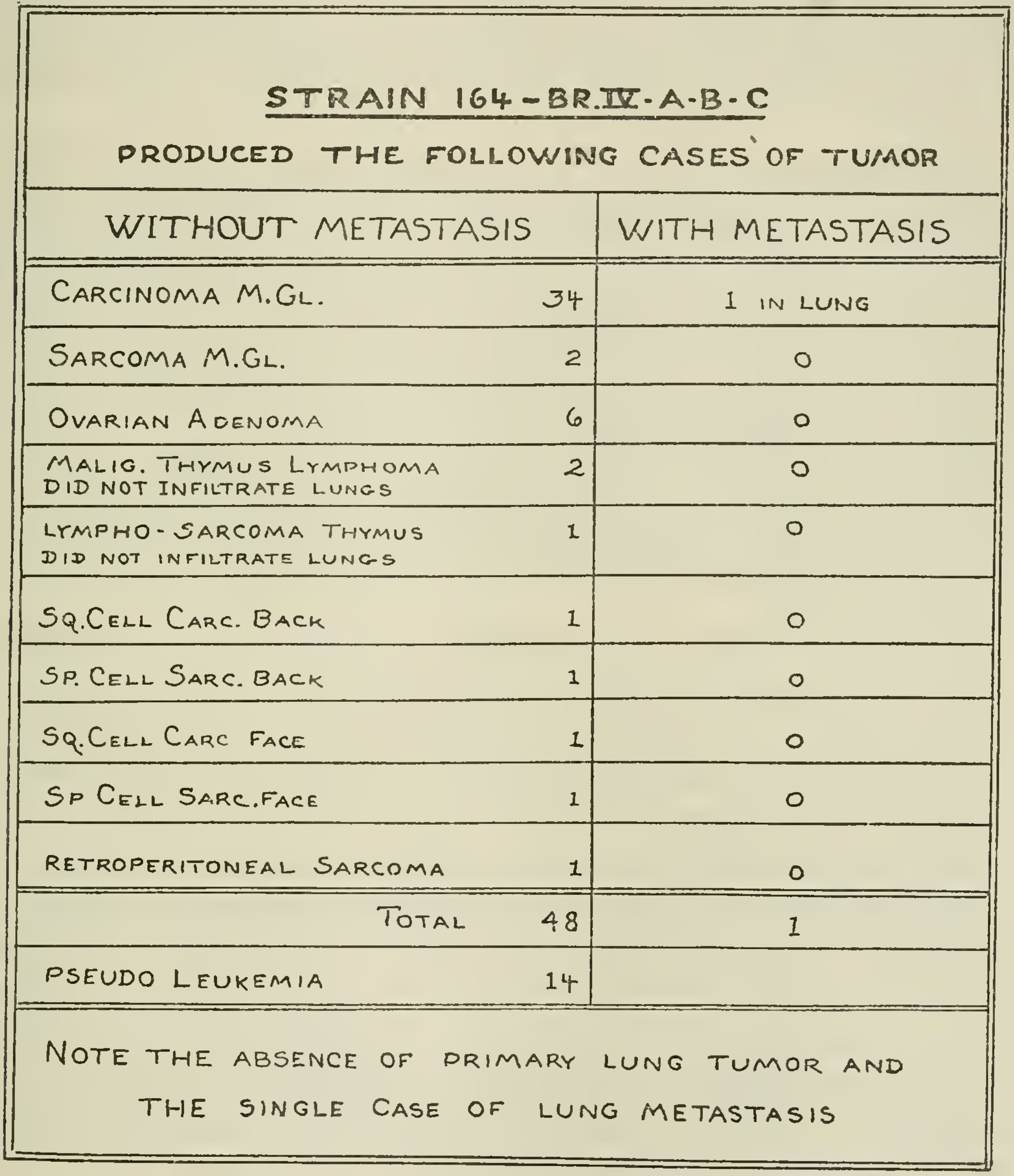


CHART 11

Chart 12 gives a summary of strain 164, branch IV, families A, B, and C. It is shown in summary only in order to get the data within a single chart. These branches of the family showed 48 primary tumors, all but one of them highly malignant, with only one case of lung metastasis, in female 10574, a member of line C. Note the marked absence of lung tumors primary and secondary, although lung tumors are second in frequency of all mouse tumors.

The strain also showed 13 cases of pseudoleukemia and one of leukemia. Here again, it is most interesting to note that the six cases of pseudoleukemia which occurred in line B showed not the slightest infiltration in the lungs. The malignant thymus tumors in this line of the family also failed to infiltrate the lungs, although they went extensively into the chest wall and mediastinal glands. The seven cases of pseudoleukemia which fell in line C of this strain, showed a very slight infiltration of the lung. This is the same line which showed one case of lung metastasis.



STRAIN 164-BR.IV-A-B-C

PRODUCED THE FOLLOWING CASES OF TUMOR

WITHOUT METASTASIS		WITH METASTASIS
CARCINOMA M.GL.	34	1 IN LUNG
SARCOMA M.GL.	2	0
OVARIAN ADENOMA	6	0
MALIG. THYMUS LYMPHOMA DID NOT INFILTRATE LUNGS	2	0
LYMPHO-SARCOMA THYMUS DID NOT INFILTRATE LUNGS	1	0
SQ. CELL CARC. BACK	1	0
SP. CELL SARC. BACK	1	0
SQ. CELL CARC FACE	1	0
SP CELL SARC. FACE	1	0
RETROPERITONEAL SARCOMA	1	0
TOTAL	48	1
PSEUDO LEUKEMIA	14	

NOTE THE ABSENCE OF PRIMARY LUNG TUMOR AND THE SINGLE CASE OF LUNG METASTASIS

CHART 12

We have then, in this strain, lines A and B, a marked illustration of the failure of the lungs to yield at all to tumor, primary or secondary. In other words, the lungs of this family do not show neoplastic proliferation, nor do they accept neoplastic secondaries or leukemic growth in spite of the certain and marked presence of the ordinary provocation for such proliferation. In line C of the strain, a slight tendency of the lung to proliferation is present.

This strain was derived by the hybridization of an absolutely nontumorous strain of house mice, in my hands many years, strain 358, with albino strain 146, which carried many tumors, and furnished some 100 per cent tumor families. This strain gives another marked illustration of the segregating out of these things; the tendency to neoplastic lung proliferation, either primary or secondary, failed to be transmitted at all to lines A and B of branch IV of the strain, and only slightly to line C. Note the striking contrast between this strain with no lung tumors, and strain 522 (chart 15) showing 100 per cent lung tumors (p. 167).

These four strains represented in charts 9 to 12 inclusive (which are perfectly typical) furnish both positive and negative evidence of the segregating out of different types and locations of tumors, and their transmission as such, whether as primary or as secondary neoplasms.

They furnish therefore evidence that *in certain strains there is a tendency for tumors to metastasize into certain organs, whereas in other strains, tumors of the same type in the same organ, even when they are of older and of larger growth, fail to metastasize into those organs.* Moreover in these strains, leukemia and pseudoleukemia tend to infiltrate the same organs which show primary and secondary neoplasms, and fail to take hold of the same organs which do not yield to primary or secondary tumors.

In this connection, it is interesting to analyze the results of various experiments to test the effect upon metastasis formation of making exploratory incisions into tumors, of massaging tumors, etc. Let me indicate what would be a complete biological control in such experiments.

TWO ILLUSTRATIONS OF COMPLETE BIOLOGICAL CONTROL.

I

Strain 164 branch C of the Slye stock, during its twelve years of existence has consistently yielded 0.01 per cent of lung tumors primary and secondary, leukemic and pseudoleukemic infiltration of the lung, and infiltration into the lungs from malignant thymus tumors.

If now we use tumorous mice from strain 164 branch C, and excise for diagnosis pieces of their tumors or massage their tumors or otherwise manipulate them, and if we find at autopsy that the group of mice whose tumors have been manipulated show little or no higher percentage than 0.01 per cent of lung tumor metastasis, and that the group which is not manipulated runs true to this 0.01 per cent lung tumor metastasis, we shall have a perfect control, and shall be justified in saying, without qualification, that excising a piece of tumor for diagnosis or massaging the tumor, or otherwise manipulating it, had no effect upon the production of lung tumor metastasis.

II

Strain 65 branch II B has during its twelve years of existence produced 35 per cent of lung tumor metastasis.

If now we repeat these same experiments with this strain, and find at autopsy that the group whose tumors have had a piece excised for diagnosis or which have been in any other way manipulated, show little or no increase in lung tumor metastasis over 35 per cent, and if the unmanipulated group runs true to this percentage of lung tumor metastasis, we shall have a perfect biological control of the experiments, and shall be justified in saying without qualification that the excision of a piece of tumor for diagnosis or any other form of manipulation had no effect upon the production of lung tumor metastasis.

THREE ILLUSTRATIONS OF THESE EXPERIMENTS WITH INADEQUATE BIOLOGICAL CONTROL

I

Strain 164 branches A and B have during their existence for twelve years, without exception of any sort, yielded 0 per cent of lung tumors primary or secondary even where tumor emboli were present in the lungs, 0 per cent of leukemic or pseudoleukemic infiltration into the lungs, and 0 per cent of infiltration into the lungs by extension from thymus, diaphragmatic, or other adjacent malignant tumors.

If now we repeat these same experiments with these branches of the strain, we shall find at autopsy 0 per cent of lung tumor metastasis in every group of mice, whether or not we excise a piece of tumor, or massage the tumor, or otherwise manipulate it. That is, a strain whose lungs have been proved not to yield to tumor emboli is not a strain fitted to show the effect of these or any other types of manipulation.

II

Strains 139, 522, 73, branch B, etc., have during their many years of existence without exception furnished 100 per cent of lung tumors, primary and secondary, infiltration into the lungs by leukemia and pseudoleukemia, and extension infiltration into the lungs from malignant thymus and other adjacent tumors.

If now we repeat these same experiments with these or any other 100 per cent lung tumor strains, we shall find at autopsy an exceedingly high percentage of lung tumor metastasis, probably 100 per cent in every group whether excised or not. That is, a strain which for twelve years has shown 100 per cent of lung tumor is not a strain fitted to show the effect upon lung metastasis of this or any other type of manipulation.

III

Groups of mice even when purchased from one dealer are mixed lots, secured from many divergent sources, and wholly unanalyzed as to tumor potentiality. They may include some individuals from 0 per cent lung tumor strains, some from 100 per cent, some from 35 per cent, etc. If we take such groups of mice and repeat these same experiments, we shall have *no biological control,* and we shall not be justified in saying whether the lung tumor metastases found at autopsy were or were not caused by the excision of a piece of tumor for diagnosis, or by massage, or whatever form of artificial manipulation may have been used.

It is greatly to be deplored that the vast majority of experimental studies in animal pathology and bacteriology have not been and are not as yet being conducted with animals whose hereditary potentialities have been studied. It is being taken for granted that pathological conditions found in the animal after experimental procedure, are the result of such experimental procedure. No allowance is being made for heredity.

Until stocks of animals to be used in such experiments, have been thoroughly tested out as to their inherited potentialities, such experiments are lacking in adequate control, as the factor of heredity is not being considered, although it is tremendously potent. Heredity alone would be sufficient to give the exact results attained in countless experiments which have been conducted without any effort to study or control the heredity factor. Moreover, most of these studies in metatasis production by the artificial manipulation of the primary tumor have been conducted with grafted tumors. As has frequently been stated in the series of cancer studies from this laboratory, the biological difference between spontaneous and grafted tumors is so fundamental and so complete, that the behavior of grafted tumors has practically no bearing upon the behavior of spontaneous tumors.

POINT III

Individuals with secondary tumors in any given organ seem to be as potent as individuals with primary tumors in the same organ to transmit by heredity primary tumors in that organ.

Note chart 13, showing part of strain 304 with ancestry. Here two successive generations of females (529 and 467) with second-

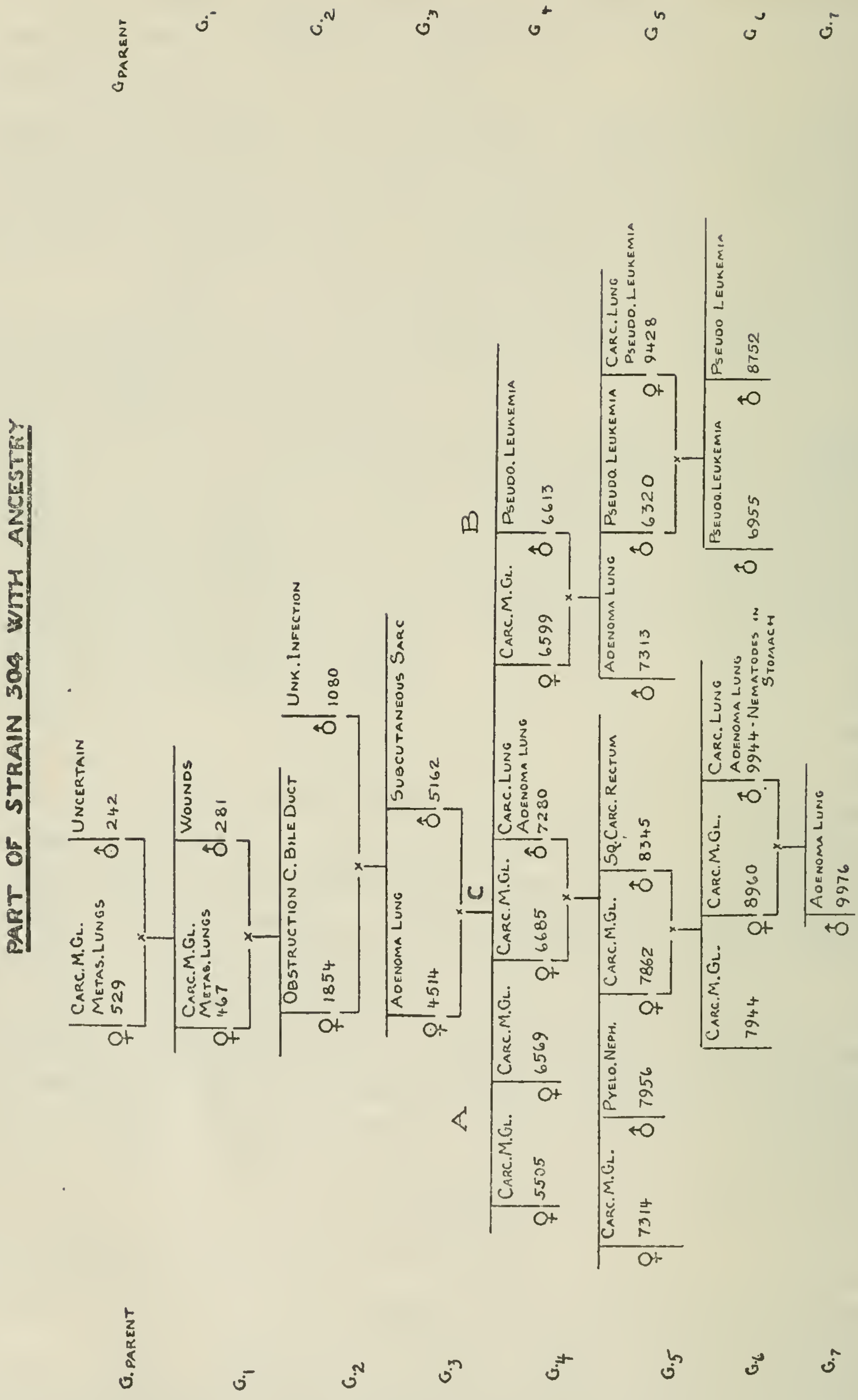

PART OF STRAIN 304 WITH ANCESTRY
G. PARENT
G.1
G.2
G.3
G.4
G.5
G.6
G.7
♀ Carc.M.Gl. Metas.Lungs 529
♂ Uncertain 242
♀ Carc.M.Gl. Metas.Lungs 467
♂ Wounds 281
♀ Obstruction C. Bile Duct 1854
♂ Unk. Infection 1080
♀ Adenoma Lung 4514
♂ Subcutaneous Sarc 5162
A
C
B
♀ Carc.M.Gl. 5505
♀ Carc.M.Gl. 6569
♀ Carc.M.Gl. 6685
♂ Carc.Lung Adenoma Lung 7280
♀ Carc.M.Gl. 6599
♂ Pseudo.Leukemia 6613
♀ Carc.M.Gl. 7314
♂ Pyelo.Neph. 7956
♀ Carc.M.Gl. 7862
♂ Sq.Carc.Rectum 8345
♂ Adenoma Lung 7313
♂ Pseudo.Leukemia 6320
♀ Carc.Lung Pseudo.Leukemia 9428
Carc.M.Gl. 7944
♀ Carc.M.Gl. 8960
♂ Carc.Lung Adenoma Lung 9944-Nematodes in Stomach
♂ Pseudo.Leukemia 6955
♂ Pseudo Leukemia 8752
♂ Adenoma Lung 9976
x

CHART 13

ary lung tumors, have originated a strain which, in line C, is producing a 44.4 per cent primary lung tumor strain. The combination of both lines B and C shows 33.3 per cent of primary lung tumors. Note the 100 per cent pseudoleukemia family being extracted in branch B, in all of which the lungs were infiltrated extensively.

Again, in chart 14, strain 338 branch VI, a 100 per cent lung tumor strain is being extracted from the original mating of female 5417, with a *secondary* lung tumor, with male 7736, with a primary lung tumor; that is, the metastatic lung tumor is behaving like a primary lung tumor in helping to produce a 100 per cent lung tumor strain, primary and secondary.

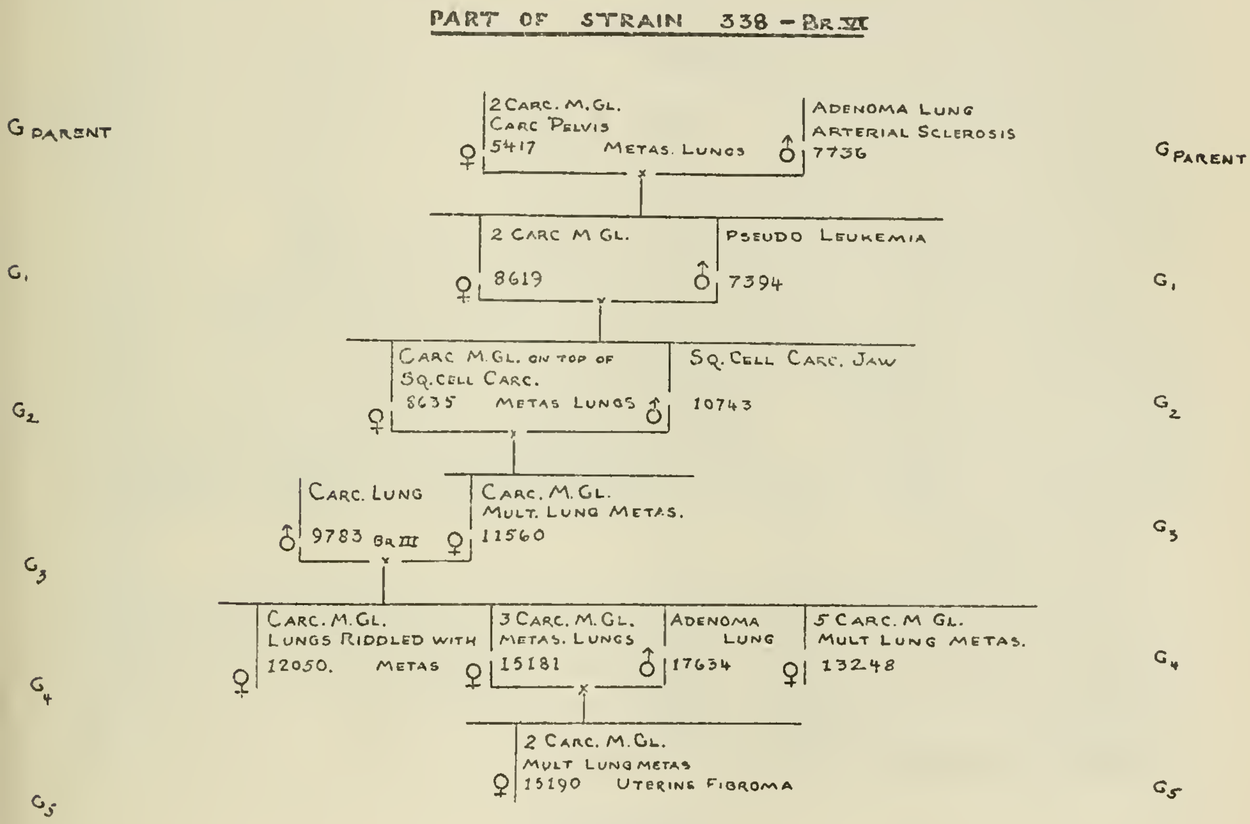


CHART 14

Refer again to chart 1 (page 142), showing part of strain 215, and note how female 630 (parent of branch B) with a metastatic carcinoma of the liver, produced three straight generations of primary liver adenoma (not yet malignant) females 8871, 11237, and 12207, as well as a primary malignant adenoma of the liver in male 2327. Note also how her secondary lung tumor was followed by a primary lung tumor in female 823 (G_3).

Refer again to chart 2 (page 145), showing strains 48 and 292, and note how the secondary sarcoma of the kidney in parent female 3, resulted in primary sarcomas of the kidneys in females 26, 348, and 396. Refer again also to chart 3 (page 147), strain 65, and note how secondary lung tumor in the parent female 5738, introduced primary lung tumors in the strain, in females 1253, 8749, 11512, and 13386, and in male 11832. Note that the thymus sarcoma in female 9888 was markedly infiltrating the lungs, while in strain 164 Br. IV, lines A and B, which did not produce lung tumors of any sort, either primary or secondary and in which leukemia and pseudoleukemia failed to infiltrate the lungs, the thymus tumors also failed to infiltrate the lungs.

Note also how in chart 15, strain 522, female 5636, with a secondary lung tumor (G_2), mated with male 8102 with a primary lung tumor, produced a 100 per cent primary lung tumor strain (center of the chart). The pseudoleukemia in this 100 per cent lung tumor strain, viz., female 14033, showed marked infiltration in the lungs.

From these charts, viz., 13 to 15 inclusive, and also from charts 1, 2, and 3 (which are perfectly typical) it is evident *that individuals with secondary tumors in any given organ seem to be as potent as individuals with primary tumors in that organ, to transmit by heredity, primary tumors in that organ.*

DISCUSSION

We have here, in the charts presented in this report, biological evidence of the nature of cancer heredity. It is well to bear in mind that until the facts of pathology have been based upon the more fundamental biological facts, it will never be possible to get a complete explanation of the pathological behavior of tissues.

Let me repeat at this point, what I already have stated frequently in previous publications: the materials used for this study are individuals analyzed as to their hereditary poten-

PART OF STRAIN 522 WITH PARTIAL ANCESTRY

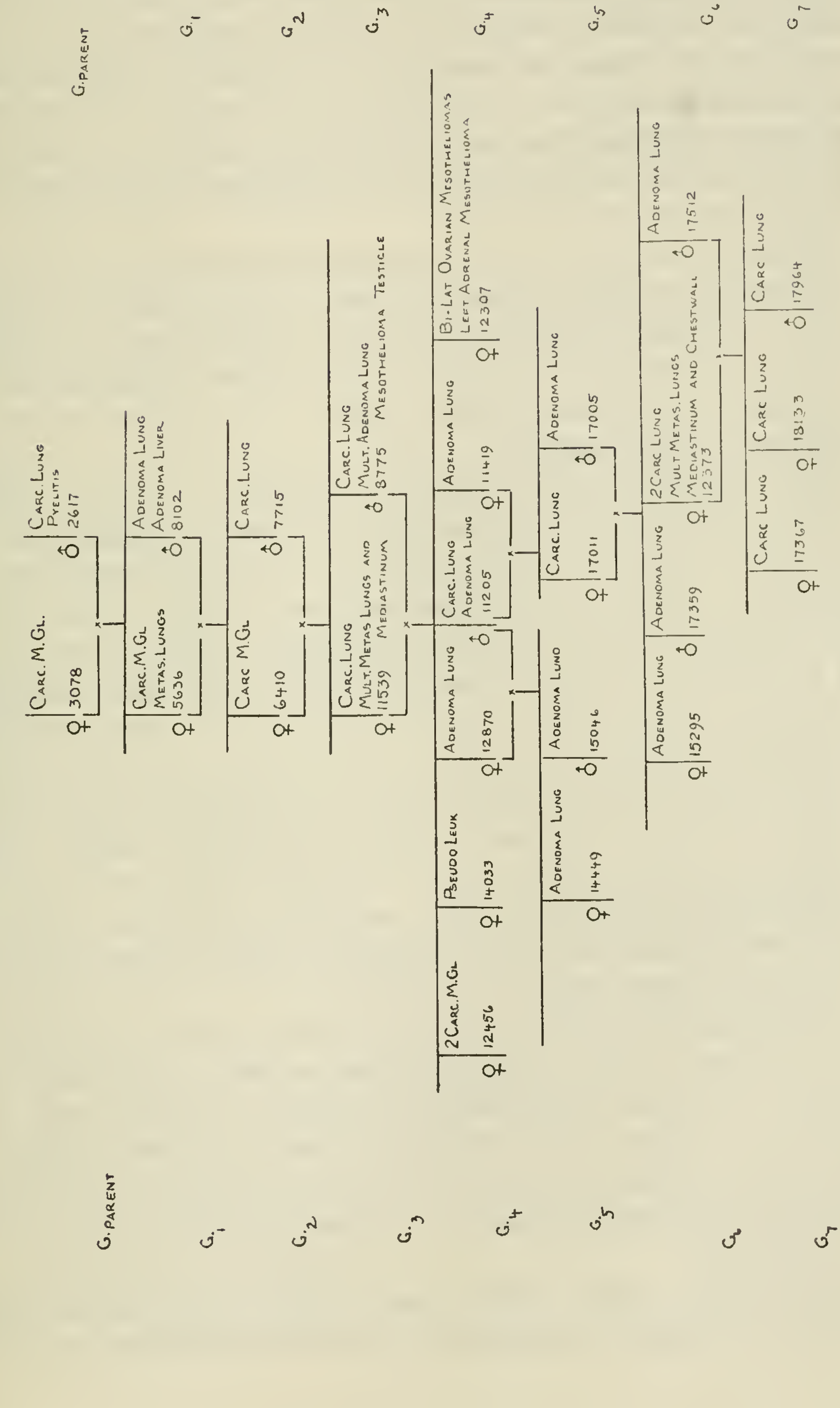

G. PARENT
G.1
G.2
G.3
G.4
G.5
G.6
G.7
♀ 3078 Carc. M. Gl.
♂ 2617 Carc. Lung Pyelitis
♀ 5636 Carc. M. Gl. Metas. Lungs
♂ 8102 Adenoma Lung Adenoma Liver
♀ 6410 Carc M. Gl.
♂ 7715 Carc. Lung
♀ 11539 Carc. Lung Mult. Metas Lungs and Mediastinum
♂ 8775 Carc. Lung Mult. Adenoma Lung Mesothelioma Testicle
♀ 12456 2 Carc. M. Gl
♀ 14033 Pseudo Leuk
♀ 12870 Adenoma Lung
♂ 11205 Carc. Lung Adenoma Lung
♀ 11419 Adenoma Lung
♀ 12307 Bi-Lat Ovarian Mesotheliomas Left Adrenal Mesothelioma
♀ 14449 Adenoma Lung
♂ 15046 Adenoma Lung
♀ 17011 Carc. Lung
♂ 17005 Adenoma Lung
♀ 15295 Adenoma Lung
♂ 17359 Adenoma Lung
♀ 12373 2 Carc Lung Mult Metas. Lungs Mediastinum and Chestwall
♂ 17512 Adenoma Lung
♀ 17367 Carc Lung
♀ 18133 Carc Lung
♂ 17964 Carc Lung

CHART 15

tialities by every possible test. The tumors are all spontaneous, arising without interference of any sort except that of selective breeding. By the process of selective breeding alone, using analyzed individuals as material, it is possible to extract strains in which the lungs do not react with either primary or secondary neoplastic proliferation or leukemic infiltration; or strains in which the liver does not so react, or the kidneys, or the mammary gland tissue, etc.

Etiological meaning. The fact that both primary and secondary tumors of a given organ or organs tend to occur in the same strains, and fail to occur at all in other tumor strains, indicates beyond question that *heredity is a strong factor in determining not only where the primary tumors shall occur, but also where the secondary tumors shall occur.*

The consequent fact, that it is possible to extract strains where only certain organs shall furnish both the primary and the secondary neoplasms, and shall yield to leukemia and pseudoleukemia; and to extract other strains in which these organs never furnish either primary or secondary neoplasms nor yield to leukemic or pseudoleukemic invasion, and that secondary tumors are just as potent as primary tumors in the transmission of primary tumors in any given organ, shows beyond a doubt that the thing which is transmitted in the heredity of cancer is the tendency of a given organ or organs to yield to cancer. This inherited tendency of an organ or organs to yield to cancer is manifested, whether the lesion is primary in that organ, or whether cells from the primary growth lodge in that organ and form a secondary lesion.

The negative evidence on this point is just as conclusive, that is, that in strains from which tumors of a certain organ or organs have been eliminated by heredity, the cells from the primary neoplasm fail to take hold, even when they lodge in such organ, and do not form a secondary growth; and even when a tumor completely surrounds an organ, it fails to penetrate it by extension.

Now what do these things mean? They mean that the tendency to carcinoma segregates out and is transmitted as such;

that the tendency to sarcoma or adenoma, etc., segregates out and is transmitted as such; that a strong tendency to the location of one or more of these types of tumor in a specific organ, such as the liver, the lung, the kidneys, the mammary glands, etc., is transmitted, owing to the segregating out of a peculiar type of tissue in these organs, which will respond in a neoplastic manner to lesions of any kind which furnish a chronic irritation of not too destructive a type. That is, the *tissues of these organs are of the same nature as the ancestral organs from which they are derived. There is a specificity of tissue type from liver to liver, or from kidney to kidney, etc., which will make those organs react in a given way to a given type of irritation.*

Now with each of these things being transmitted as such, it is possible for a parent female with a sarcoma-carcinoma of the mammary gland, a malignant adenoma of the liver, and a secondary sarcoma of the kidney (like female 3) to transmit to the strains derived from her, a tendency to:

1. Carcinoma of the mammary gland.
2. Sarcoma of the mammary gland.
3. Adenoma of the mammary gland.
4. Carcinoma of the liver.
5. Sarcoma of the liver.
6. Adenoma of the liver.
7. Carcinoma of the kidney.
8. Sarcoma of the kidney.
9. Adenoma of the kidney, or any combination of these nine.

If she is mated with a male, either himself having a lung and a mediastinal tumor, or being heterozygous to these locations of tumor (as in the case of male 360), the resulting strains will show:

1. Carcinoma of the mammary gland.
2. Sarcoma of the mammary gland.
3. Adenoma of the mammary gland.
4. Carcinoma of the liver.
5. Sarcoma of the liver.
6. Adenoma of the liver.
7. Carcinoma of the kidney.
8. Sarcoma of the kidney.

9. Adenoma of the kidney.
10. Carcinoma of the lung.
11. Sarcoma of the lung.
12. Adenoma of the lung.
13. Carcinoma of the mediastinum.
14. Sarcoma of the mediastinum.
15. Adenoma of the mediastinum, or any combination of these tumors.

The neoplastic growth may be primary in any one or more of these organs, according to where there is occasion for the primary lesion to occur, and there may be secondary growths in any one or more of these organs, according to where the secondary lesions occur.

Now note that this is exactly what we get from the matings of female 3 (with a sarcoma-carcinoma of the mammary gland, a malignant adenoma of the liver, and sarcoma metastasis in the kidney) with male 360, proved heterozygous to lung and mediastinal tumors. Note especially charts 1 and 2 where the strains were derived from the same original mating of female 3 with male 360; and charts 4, 5, 6, 7, 8, and 9, showing ancestry and strains derived from the mating of female 3 with male 30.

It should not be a matter for surprise that there is an inheritance of a specific type of liver tissue, or kidney tissue, etc., which will tend to respond in a given way to a given irritation or a given lesion. Similar facts of inheritance, such as a tendency to grow tall, with the proper degree of nourishment, etc., are easily accepted. That also is the inheritance of tissues which tend to respond in a given way to a given stimulus. There is here also a certain specificity, as, for example, the tendency to longness in the trunk and shortness of the limbs; or of shortness of the trunk and longness in the limbs, so that a given figure tends to predominate in a given family, just as given types and locations of neoplasms tend to predominate in a given family. Again, it is an accepted expectation that there should be a given nose shape, or shape of lips, or contour of head, inherited within a family; i.e., a specificity of nose tissue, etc., which will insure its growing to a given shape, etc.

This specificity of organ tissue, which shall insure its reacting in a given neoplastic way, to a given chronic irritation, is exactly what we find to be the nature of cancer heredity; and it is obvious that it will make no difference in the nature of the reaction of such specific organ tissue, whether the lesion is primary or secondary.

Heredity, therefore, of a specific type of organ tissue, is here shown to be the fundamental influence in determining the incidence and location of metastatic neoplasms, as well as those of primary neoplasms.

In regard to the relative infrequency of secondary tumors in this stock, the biological evidence is as follows: Many of these strains have been made by hybridizing a tumorous individual with a proved non-tumerous individual. This, of course, is done to test for the Mendelian behavior of cancer in heredity. Now, with the tendency to carcinoma, sarcoma, etc., segregating out, and a specific type of organ tissue segregating out and being transmitted as such, it is evident that any given mouse may inherit a tendency to only one type of tumor, and in only one organ. Other organs, therefore, in such a mouse, refuse to yield to cancer, even where emboli are present in such organs. Many such cases have been pointed out as shown in the charts in this report.

Individuals into whose ancestry (as is the case in much human heredity) an indiscriminate amount of cancer had been bred would show a correspondingly higher percentage of metastasis.

SUMMARY

These studies in the metastasis behavior of spontaneous tumors demonstrate the following facts:

1. In any given strain, the metastatic tumors (where there are any) tend to occur in exactly the same organs in which the primary tumors of that strain occur.

2. In certain strains, there is a tendency for tumors to metastasize in certain organs; whereas in other strains, tumors of the same type in the same organ, even where they are of older and of larger growth, fail to metastasize into those organs.

3. Leukemia and pseudoleukemia, occurring in tumor strains, pick out predominantly the same organs for infiltration which show the primary and secondary tumors of that strain.

4. Tumors do not even invade by extension the organs from which primary and secondary neoplasms have been eliminted by heredity.

5. Individuals with secondary tumors in any given organ, seem to be as potent as individuals with primary tumors in the same organ, to transmit by heredity, primary tumors in that organ.

Therefore:

6. Heredity is a strong factor in determining not only where the primary tumors of a strain shall occur, but also where the secondary tumors shall occur.

7. Heredity is a strong factor in determining what organs of a strain shall yield to the invasion of leukemia and pseudoleukemia.

8. The thing which is transmitted in the heredity of cancer is the tendency of an organ or organs to yield to cancer. This tendency is manifested whether the lesion is primary in that organ, or whether cells from the primary growth lodge in that organ and form a secondary lesion.

9. The tendency to sarcoma, carcinoma, adenoma, etc., segregates out and is transmitted as such.

10. A strong tendency to the location of one or more types of tumor in a specific organ or organs, such as the liver, kidney, pancreas, mammary gland, etc., is transmitted, owing to the segregating out of a peculiar type of tissue in these organs, which will respond in a neoplastic or leukemic manner to lesions of any kind which furnish a chronic irritation of not too destructive a type.

11. That is, there is a specificity of tissue type, from organ to organ in a strain, which will make these organs react in a given way to a given type of irritation.

12. It is, therefore, possible for ancestry to transmit to its posterity every possible combination of the neoplastic or leukemic tendencies which they carry either actually or potentially.

13. This specificity of tissue type in organs, which will insure its reacting in a given neoplastic manner or accepting leukemic deposits, in response to a given chronic irritation, is what we find to be the nature of cancer heredity, and it is obvious that it will make no difference in the nature of the response of such specific organ tissue, whether the lesion is primary or secondary in that organ or organs.

Heredity, therefore, of a specific type of organ tissue, is here shown to be the fundamental influence in determining the incidence and location of secondary tumors and of leukemia and pseudoleukemia, just as it is in determining, the incidence and location of primary neoplasms.

It is also pointed out that any apparent testimony of the frequent occurrence of secondary tumors in man in tissues where primary tumors rarely occur, would be of no help here, as no human strain has ever been even partially analyzed, and no right conclusions regarding heredity can be drawn except from analyzed strains.

It is also suggested, that until stocks of animals to be used in pathological and bacteriological experiments, have been thoroughly tested out as to their inherited potentialities, such experiments will be lacking in any adequate control, since heredity is not considered although it is tremendously potent.

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(8) Bashford, et. al: Third Sci. Report, Imperial Cancer Research Fund, 1908, 322.
(9) Woglom: J. Cancer Res., 1918, iii, 47.
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PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH

FOURTEENTH ANNUAL MEETING

Held in Cleveland, Ohio, March 24, 1921

1. Report of the Council

The meeting of the Council was held at the Hotel Statler in Cleveland, Ohio, on the evening of Wednesday, March 23, 1921.

The following members were present: Dr. Robert H. Greenough, president; Dr. Francis C. Wood, and Dr. William H. Woglom. Absent, Dr. James B. Murphy, Dr. E. R. LeCount, Dr. Willy Meyer, and Dr. James Ewing. As four members of the Council are required for a quorum, business was transacted temporarily with the idea of obtaining subsequently the vote of another councillor. Dr. Murphy's vote sustaining the action of the Council was subsequently received by mail.

The treasurer's report was read and accepted.

The following officers were elected to serve for the ensuing year: Dr. James B. Murphy, president; Dr. Willy Meyer, vice-president; Dr. William H. Woglom, secretary and treasurer (re-elected).

Dr. H. Gideon Wells was elected Councillor to succeed Dr. E. R. LeCount, whose term of office expired.

The present Council, therefore, with the years of retirement, is as follows:

Dr. F. C. Wood, 1922
Dr. James B. Murphy, 1923
Dr. William H. Woglom, 1924
Dr. Robert B. Greenough, 1925
Dr. Willy Meyer, 1926
Dr. James Ewing, 1927
Dr. H. Gideon Wells, 1928

The present Editorial Board was continued in office. It is composed of

Editor, Dr. Woglom
Associate Editor, Dr. Prime
Dr. Bloodgood
Dr. Wells
Dr. Loeb
Dr. Ewing
Dr. Tyzzer

The following resignations were accepted:

Dr. C. L. Alsberg	Associate
Dr. D. B. Phemister	Associate
Dr. Robert G. Leconte	Associate
Dr. F. P. Gay	Active

There has been one death during the year—Dr. H. H. Janeway, an active member, who died on February 1, 1921.

The following gentlemen were elected to membership:

Active

Dr. Herbert U. Williams, Univ. of Buffalo
Dr. David Marine, Montefiore Home and Hospital
Dr. William Ophuls, San Francisco, Calif.
Dr. H. T. Karsner, Cleveland, Ohio
Dr. Wm. Bayard Long, New York
Dr. Leo Buerger, New York
Dr. James H. Wright, Mass. General Hospital
Dr. Carl V. Weller, Ann Arbor, Mich.
Dr. Georgine Luden, Mayo Clinic
Dr. Henry Albert, Univ. of Iowa
Dr. Eugene L. Opie, Washington Univ. Sch. of Med.

Associate

Dr. John G. Clark, Philadelphia, Penn.
Dr. Stuart Graves, Louisville, Ky.
Dr. Harris P. Mosher, Marblehead, Mass.
Dr. Kenneth T. Taylor, New York
Dr. G. R. Minot, Boston
Dr. Seth Milliken, New York
Dr. John L. Yates, Milwaukee, Wis.
Dr. Charles Norris, Chief Med. Examiner, New York
Dr. D. Crosby Greene, Boston
Dr. F. S. Mandlebaum, New York
Dr. Hugh H. Young, Johns Hopkins Hospital
Dr. Henry A. Christian, Peter Bent Brigham Hospital
Dr. Otto V. Huffman, New York
Dr. L. Duncan Bulkley, New York
Dr. Joseph A. Blake, New York
Dr. Ward J. MacNeal, Forest Hills, N. Y.
Dr. Channing C. Simmons, Boston, Mass.
Dr. Otto Krehbiel, New York
Dr. Julius Rosenstirn, San Francisco, Calif.

The application of Mr. Donald C. A. Butts, who has not yet published any articles on cancer, was laid on the table, and the application of Dr. Rex Duncan was postponed.

SCIENTIFIC SESSION

A letter from Dr. H. J. Conn, Chairman of the Committee on Bacteriological Technique, of the Society of American Bacteriologists, calling on biologists to coöperate with that Society in helping to secure a reliable domestic source of stains was brought to the attention of the Association of Cancer Research at its scientific meeting on March 24, by the President, Dr. Robert B. Greenough.

It was moved by Dr. Wood and seconded by Dr. Gaylord that the Association encourage the manufacture of American dyes. The motion was carried.

2. Report on the Journal of Cancer Research

Dr. F. C. Wood (New York):

SUMMARY

It has seemed to the Council a pity to allow the Journal of Cancer Research to lapse, inasmuch as the volumes so far printed contain most important papers on cancer research, and when a journal ceases publication the libraries put the bound volumes on the back shelves and no one ever sees them again, unless specially inquired for. The Association has resigned all responsibility for the Journal of Cancer Research and the Crocker Fund, Columbia University, has assumed it. There will be no change in form or publishers at the present time. The Crocker Fund will have to meet an annual deficit in the cost of publication and I hope the members of the Association will do all they can to increase our subscription list, because unless this is done the Crocker Fund will have to continue the present practice of the Editorial Board and charge pretty heavily for cuts and reprints.

If we could get an additional three or four hundred subscribers it would greatly reduce the cost of publication to the individual writers.

DISCUSSION

Dr. Robert B. Greenough (Boston): The Council felt that the Crocker Fund is coming to the rescue of the Association in the matter of the Journal of Cancer Research and that there is a distinct obligation upon this Association that the Journal should not be abandoned; for much of the work in the past six or seven years in this country has been published in this Journal. If abandoned, the material in the earlier editions is lost and not readily accessible to new investigators. It is thus an obligation to the contributors that the Journal should be continued, and apparently only by means of assistance from the Crocker Fund can this be done. For these reasons the offer of the Crocker Fund was accepted.

3. Further Studies in Radiation Dosage

Dr. F. C. Wood and Dr. Frederick Prime (New York):

SUMMARY

Dr. Wood showed a series of lantern slides illustrating the fact that, contrary to statements emanating from Germany, there is no destructive carcinoma or sarcoma dose; that is, no fixed amount of x-ray can be assumed to destroy the cells of any one tumor, for apparently the dosage differs greatly both in man and animals for the same microscopic type of tumor.

As the charts showed, in animal tumors the required dosage is from 2 to 8 erythema doses, and the sarcomata vary in resistance as well as the carcinomata. One interesting thing is the greatly delayed appearance of tumors at a point where the cells received a sublethal dose. In a mouse tumor which ordinarily appears within a week and gains a fair size in two weeks, the appearance may be delayed for a month or more and the tumor grow very slowly afterward. If a transplantation be made from this tumor into another animal the growth rate will rapidly approximate normal. Inasmuch as two or three months in a mouse's life is equivalent to the same number of years in a human being, the question must be raised whether we are not going to see late recurrences, three or four or five years after the symptomatic cure of malignant tumors in man, when such symptomatic cure is obtained by either radium or x-ray.

Most of our experiments have been made by raying the tumor particles *in vitro* and then transplanting them; if the tumor be rayed in the mouse the animal is killed in a few days by the radiation. If the tumor be immediately transplanted it grows in the new host, unless it has been given a lethal dose. This lethal dose is approximately 10 to 20 per cent more than when the cells are exposed *in vitro.*

DISCUSSION

Dr. E. T. Bell (Minneapolis): What is your opinion of the treatment of cancer of the cervix uteri with very high voltage apparatus such as is now in use in Germany?

Dr. F. C. Wood: Replying to Dr. Bell's inquiry, whether rays from new high voltage machines are more effective than those from the older type of apparatus, Dr. Wood said he had not found that there is any increase in the destructive action of rays given off by tubes running at 180,000 volts as compared with those running at 120,000 volts, measuring peak voltages between 12.5 cm. spheres. In all his tests there had been no question of absorption or of scattered radiation, the tumor having been directly exposed to the rays. The advantage of the high voltage machines lay in the greater penetration which can be obtained, but there is no reason to assume that they would be any more effective on superficial growths for equal x-ray dosage. Reports received recently from German clinics seem to indicate that some of the published results are greatly exaggerated.

In reply to an inquiry from Dr. Greenough regarding stimulation effects, Dr. Wood said that he had not noticed any increase in growth rate of the tumors, unless very small doses were given; and that the tumors which had been greatly slowed at their first transplantation by large doses, grow at their regular rate in the second transplantation.

In response to an inquiry as to the exact mechanism of the effect of x-ray on the cell, and whether the connective tissue does not play an important part in the destruction of the tumor cell, Dr. Wood replied that he had not the slightest idea of the nature of the changes causing destruction of the cells by x-ray or radium. All we know is that the division mechanism is interfered with, and that cells in a resting phase are much more resistant to radiation than those in mitosis. Experience with animals proves that it takes very large doses to kill all the cells of a tumor; those in the center of the growth are killed partially by direct action and partially by the thrombosis which occurs early in the smaller capillaries. The periphery of the tumor, however, is, as a rule, better nourished and there the cells require maximum dosage. An absolute cure requires the killing of every cell during a single exposure, for if divided doses be given, some cells may recover from the radiation effects. Dr. Wood had not observed that connective tissue is capable of destroying cancer cells. It is perfectly possible that such cells might be inclosed in dense scar tissue and remain quiescent for a considerable period, but there is a possibility that they might again begin to grow years after their inclusion. This had been frequently observed in human tumors in the late recurrences in operation scars. Dr. Wood had seen some such recurrences five, six, seven, or eight years after operation.

In response to an inquiry as to the voltage used, Dr. Wood said that this was 120,000 volts, peak, measured between 12.5 cm. spheres. The tumor fragments used in vitro were about 1 to 2 mm. in diameter. If whole tumors be used, or if the tumor be exposed in the mouse, the superficial layers nearest the tube will be killed, while the deeper layers are not. At this voltage, 10 cm. of tissue absorbed 50 per cent of x-ray incident on the surface; this 50 per cent includes not only directly transmitted rays but also scattered ones. His own experiments had all been devised to avoid the complications brought into the problem by scattering and absorption, the effect of which can be easily determined by an ionization chamber; this varies in human cases with the individual, because of the varying depth of the tumors. Dr. Wood's dosage was obtained in a way which made it independent of such effects.

Dr. William Duane (Boston): The difference between the dosage measured by Dr. Wood and that reported from Germany, may be due to scattering. According to recent reports, at a distance of 10 cm. below the skin scattering may increase the dosage $2\frac{1}{2}$ times. If these pieces of tumor be surrounded by other bits of tissue, they may perhaps receive $2\frac{1}{2}$ times as much radiation as they do under the experimental conditions described by Dr. Wood.

4. High Frequency *X*-ray Spectra

Dr. William Duane:

DISCUSSION

Dr. Wood: We all know, of course, that these experiments of Dr. Duane and the results which he has shown us, form the foundation of all our *x*-ray work. They underlie all the measurements of the amount of the *x*-ray which reaches the body; and when his results on the physical side meet mine on the biological side, we shall know the whole story. He is investigating the methods of measurement of the quality and quantity of *x*-ray which comes through our filters, while I am trying to determine how much of what he thus has measured is necessary to kill a cell. In a few years we shall know the effect of long rays or short. The one question which the practitioner has to decide is, whether the human body will stand all the radiation necessary to kill all the cancer cells. If it will not stand this amount, we cannot cure cancer with radiation; if it will, we can. I recently gave a patient something like 45 erythema doses for a melanosarcoma of the foot; the blood changes were nominal; the only effect was that the patient was somewhat nauseated. The foot was of necessity removed within twenty-four hours to avoid absorption effects from the dead tissue. This test shows that such a dose can be given on a part of the body where the rays do not penetrate important organs; but when it comes to putting large doses into the abdominal cavity it is a different question. The question of distance is also important. If we could only get a tube such that we could place the patient at a distance of a meter, conditions would be better than at present, where we work with a distance of 30 or 40 centimeters. The next step is improvement in the wattage capacity of the tube. Somebody must design a tube which will stand longer quantities of current than we can now employ. The amount of energy is very small, and exposure, therefore, amounts to six or eight hours, which is too much for a patient to stand. If we cannot contrive to shorten the exposure by use of a very powerful tube, we shall not get much farther ahead than we are at the present time.

Dr. Duane: The *x*-ray energy increases as the square of the voltage. When the voltage is doubled, four times as much radiation is obtained, thus decreasing the length of time required for exposure.

5. The Occurrence of Squamous-Cell Carcinoma in Mice

Miss Maud Slye, Miss Harriet F. Holmes, and Dr. H. Gideon Wells (Chicago): Presented by Dr. H. Gideon Wells:

SUMMARY

Review of the literature on the comparative pathology of carcinoma shows that, in general, squamous carcinomas are not so common in other animals as in man, with the possible exception of the dog, in which carcinoma of the skin is not infrequent. This infrequency in animals is probably more real than statistics indicate, since the skin carcinoma will usually be recognized, whereas the great majority of internal growths will escape attention. In horses, carcinoma of squamous character is seen occasionally about the external genitalia of both sexes.

Fowls and other birds not infrequently show carcinoma of the skin, and such growths have also been observed in fish and amphibia. A few tumors of this group have been described in rats, and squamous-cell tumors of the mouth and skin have been observed in mice as well as a few cases of squamous-cell carcinoma arising from the cardiac end of the stomach. Most of the squamous-cell carcinomas described in mice have occurred in the mammary gland, apparently arising either from the ampullae of the lacteal ducts or by metaplasia of a columnar cell carcinoma.

In 28,000 consecutive autopsies performed on mice of the Slye stock, which had been permitted to live as long as possible without any experimental manipulations whatever, we have observed the following instances of primary neoplasms of squamous or stratified epithelial structure.

Primary squamous-cell carcinoma of the skin and mouth	71
Primary basal-cell carcinoma of the skin	15
Primary keratinizing cell carcinoma of the mammary gland	56
Primary squamous-cell carcinoma of the stomach	4
Primary keratinizing cell carcinoma of the lung	1
Primary squamous-cell carcinoma of the rectum	2
Primary squamous-cell carcinoma of the vagina	1
Primary stratified carcinoma of meibomian gland	2
Primary sebaceous gland adenocarcinoma	1
	153

The squamous-cell carcinomas of mice are distinguished especially by the infrequency of metastasis and they generally show a relatively slight tendency to infiltrate, although we have observed even infiltration of the skull and spinal column. This probably depends on the fact that secondary infections usually kill the mice at an early stage of the disease. All the basal-cell carcinomas and the great majority of squamous carcinomas arose about the head, neck, and mouth, these being the sites of the greatest amount of irritation. Frequently they have arisen in healed wounds.

(A full report of this material will be published in a forthcoming number of the JOURNAL OF CANCER RESEARCH.)

DISCUSSION

Dr. Wells: In reply to a question regarding the life span of the mouse, Dr. Wells replied that it depends upon the stock. Miss Slye has a strain of Japanese waltzing mice which become senile before they are a year old. The chief cause of death in old mice is chronic nephritis.

Miss Slye: The average published life of the mouse is 1½ years, but many mice in my laboratory live to be six years old or more; the average age, however, is from three to four years. Every effort is made to keep them alive as long as possible.

Dr. Wells: I may state that these squamous-cell carcinomas in mice arise distinctly at a later age on the average than the other carcinomas. This is true in human squamous-cell carcinomas, too.

Dr. William H. Woglom (New York): In a paper published in the JOURNAL OF CANCER RESEARCH I have discussed the frequency of keratin in mammary carcinoma of the mouse, and have estimated it at about 23 per cent.

6. THE INFLUENCE OF HEREDITY IN DETERMINING TUMOR METASTASIS: STUDIES IN THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE. FIFTEENTH REPORT

Miss Maud Slye:

SUMMARY

Metastases in this stock are somewhat rare. In 29,000 autopsies, furnishing something over 4000 primary spontaneous tumors, about 19 per cent of the growths metastasized. The general testimony of those who have discussed metastasis at all, seems to be that the occurrence and location of metastasis is partly a mechanical matter, and partly a striking specificity of localization of secondary growths. This specificity they make no attempt to explain.

Ewing, speaking of metastasis in human tumors, states that in highly vascular tissues like the lip, stomach, and testicle, very small carcinomas may yield distant metastasis. But these very organs are among those whose tumors rarely metastasize in this stock, and then only locally. For example, of our carcinomas of the lip none has metastasized; of growths in the testis, one metastasized locally; while among carcinomas of the stomach three metastasized into the regional lymph-nodes only. And this in spite of the close resemblance between these neoplasms and human tumors in similar organs.

In this stock mammary gland tumors rarely metastasize into the regional lymph-nodes, in contrast to those of the human breast. Indeed, in many cases in this stock, carcinoma and sarcoma of the mammary gland can be seen growing up to the lymph-node but not invading it. On the other hand, pulmonary metastases from mammary gland carcinoma and sarcoma are common in this stock.

These divergences in the metastatic behavior of tumors of similar type and in similar organs, require some explanation other than a mere mechanical tendency of certain types of tumor to metastasize in certain locations.

Metastasis in this stock has been under observation for ten years and the data here given are based on over 29,000 autopsies.

Briefly stated, these results show conclusively: (1) In any given strain the metastatic tumors tend to occur most frequently in exactly the same organs in which the primary tumors of that strain occur. For example, if a strain is high in primary tumors of the liver, many secondary tumors will be found in that organ. Again, if a strain yields large numbers of primary lung tumors, metastasis will tend to occur in the lungs from primary tumors in almost any location. A strain high in primary kidney tumors is high also in secondary kidney tumors, etc.

(2) In certain strains there is a tendency for tumors to metastasize into certain organs; whereas in other strains, tumors of the same type in the same organ, even where they are older and of larger growth, fail to metastasize into these organs. For example, in certain strains practically every carcinoma of the mammary gland metastasizes into the lungs; I have many strains showing 100 per cent such behavior. On the other hand, in other cancer strains exactly this same type of mammary gland carcinoma, even where older and of larger growth, never metastasizes into the lungs; and I have many strains showing 100 per cent of this type of behavior. Indeed, even where tumor emboli reach the lungs in these non-pulmonary strains, the emboli fail to take hold and no tumor is formed. This is definite proof that the lung here fails to yield, even when tumor cells are present.

Again, strains which never yield primary lung tumors never yield secondary lung tumor. Strain 164 is a striking example of this, one family yielding 36 carcinomas and sarcomas of the mammary gland without one case of lung metastasis. In this family also there has never been one case of primary lung tumor.

(3) Individuals with secondary tumors in any given organ seem to be as potent as individuals with primary tumors of that organ to transmit (by heredity) primary tumors in that same organ. For example, female 3 with a primary carcinoma-sarcoma of the mammary gland, and secondary sarcoma in the kidney, transmitted primary kidney tumors to the strains derived from her, one family showing as high as 37 per cent of primary kidney tumor.

Furthermore, individuals with secondary lung tumors seem to be just as potent as individuals with primary lung tumors to transmit (by heredity) primary tumors of the lung. Thus, many strains derived from a female with a primary mammary carcinoma with metastasis in the lungs, mated with a male showing primary lung carcinoma (double lung-cancer parentage) show 100 per cent primary lung carcinoma.

(4) Etiological meaning: The fact that both primary and secondary tumors of a given organ tend to occur in the same strains and fail to appear at all in other strains, indicates that *heredity is a strong factor in determining not only where the primary tumors shall occur but also where the secondary tumors shall occur.*

This fact apparently means that the thing which is transmitted in the heredity of cancer is the tendency of an organ to yield to cancer, whether the lesion is primary in that organ or whether cells from the primary growth lodge in that organ and form a secondary lesion. Moreover, in strains from which tumors of a certain organ have been eliminated by heredity, even where cells from the primary neoplasm lodge in such organ they fail to take hold and do not form a secondary growth. It is, therefore, evident that not only primary neoplasms, but secondary tumors also, are determined in their occurrence and their location by heredity.

DISCUSSION

Dr. G. H. A. Clowes (Indianapolis): Has anybody ever attempted to determine whether any of these strains are more or less sensitive to absence of vitamines?

Miss Slye: I do not think so.

Dr. Clowes: I should think that it might be a very important factor in proliferation.

Miss Slye: I have been carrying on some dietary experiments in my own laboratory, but they are at too early a stage for me to give any data.

Dr. Wells: Dr. Wood who has investigated the development of metastases in rats subjected to exploratory incision, in order to determine the rate of metastasis after such incision, has found distinct variations among control rats of different strains, in respect to metastases. These experiments, however, were carried out with grafted tumors, whereas Miss Slye's work deals entirely with spontaneous growths.

Dr. Greenough: As I understand it, all the matings were made before it was known that female No. 3 had any tumor. Was her tumor discovered at autopsy?

Miss Slye: Female 3 had offspring both before and after the appearance of her tumor. Her mammary tumor was evident some months before her death, while the growths of liver and kidney were found at autopsy. Many of these matings are made before the tumors appear, for if one waits for the appearance of a tumor one is apt to lose the chance of offspring in a large number of cases. Mice of high cancer ancestry should be mated early. In the majority of my cancer mice I have records of offspring both before and after the appearance of tumors. In the matter of tumor inheritance it makes no difference whether the offspring precede by many months the appearance of the growth or are born after its appearance. The grandchild or great-grandchild may develop a tumor before the grandmother from whom the tumor is inherited. In tumors of the liver and kidney it is difficult to make a clinical diagnosis, though this is sometimes possible. Tumors of the lung are frequently accompanied by a peculiar condition of the eye, from which it is often possible to make a clinical diagnosis.

Dr. Clowes: Is there any relation between age and the rapidity at which tumors tend to develop and metastasize?

Miss Slye: The relation of age and tumor growth has not yet been completely worked out, but in general it is true that tumors grow more slowly in old mice, as they do in old men. Also it may be said in general that the tumors of old mice never grow to any great size, and that old mice seldom are able to support more than one tumor.

7. Proofs of the Constitutional Nature of Cancer

Dr. L. Duncan Bulkley (New York):

SUMMARY

- I. Laboratory findings
  - Negative
    - Cancer not parasitic
    - Cancer not contagious
    - No cause for cancer
  - Positive
    - Cancer cell an altered normal cell
    - Feeding experiments showing control of cancer growth
- II. Statistical evidence
  - Control of death statistics of cancer and tuberculosis, especially since 1900
  - Steady increase of cancer deaths under surgery, x-rays, and radium
- III. Bio-chemical evidence
  - Blood in cancer; early and late metabolic changes in the system before and after the development of the local cancerous lesion
- IV. Clinical
  - Opinion of many celebrated surgeons during the last 100 years, to the present time
  - Spontaneous cures of cancer reported
  - Dozens or hundreds of attested cases of benefit or cure of cancer by numerous physicians, in this and other countries

DISCUSSION

Dr. Bulkley: I hope for comments or objections, because if I am wrong I want to know it.

Miss Slye: It might interest Dr. Bulkley and the Association to know that there is a standard diet maintained in my laboratory, which is identical for tumor strains and non-tumor strains. With autopsies at 29,000 and living inhabitants numbering about 13,000, all fed on exactly the same diet, the tumor strains have yielded over 4000 spontaneous tumors, while the non-tumor strains have never yielded a single tumor. No meat is ever used in our laboratory.

Dr. W. T. Bovie (Boston): The mortality from tuberculosis in the state of Massachusetts is of interest in this connection. A curve showing the progressive decrease in mortality deviates but little from a straight line. Its course does not appear to have been changed by any of the activities of the medical profession. Such deviations from a straight line as do occur seem to be correlated with influxes of foreign people.

Dr. Wells: I am familiar with Dr. Mayo's paper on the subject of the influence of diet on gastric cancer, and it seems to me that Dr. Bulkley has mis-interpreted it. Dr. Mayo was discussing the influence of local conditions in causing carcinoma of the stomach, and not the influence of diet in its constitutional relation.

8. Relation of Heterosexual Characters to Neoplasms and Developmental Errors

Dr. Otto V. Huffman (New York): Read by title:

SUMMARY

A preliminary report on the association of heterosexual secondary sex characters with neoplasms, especially carcinomata, in patients observed by the speaker. In noting the occurrence of status lymphaticus in the clinic he observed that about ten per cent of the patients showed some degree of heterosexual secondary sex characters, especially in regard to the pubic hair and that on the chest and on the face, and in regard to the width of the chest and of the hips. He found in some of these patients such evidence of maldevelopment as a patent omphalomesenteric duct and gynecomastia. This led him to take note of the secondary sex characters of patients with neoplasms. A marked degree of heterosexuality in the secondary sex characters is not incompatible with normal sexual life and reproduction. Four married patients who developed carcinoma showed slight degrees of heterosexuality but the marriages were sterile. Another four married patients who developed carcinoma and who had some heterosexual characters did not develop their neoplasms until after their active sexual life, that is, after the age of fifty. The greatest degree of heterosexuality was observed in patients who developed carcinoma early, that is, prior to fifty years of age. This may mean that the degree of heterosexuality has some etiological significance. Between the 100% normal male and the 100% normal female we have a whole series of intergrades. Several observers have stated that these intergrades are evidently on the increase. Ordinarily they are more susceptible than the normal to acute infectious diseases, but sanitation and public health measures may be favoring them to such an extent that they mature and thereby arrive at the cancer age. From the author's observations it would seem that early cancer is relatively more prevalent among them than among the normal, but he is not yet prepared to publish comparative tables that would establish this point conclusively.

9. END-RESULT REPORTS OF CANCER OPERATIONS

Dr. Robert B. Greenough and Dr. Channing C. Simmons (Boston):

SUMMARY

While many reports of the end-results of operations for the cure of cancer have been published, and while certain standards such as the arbitrary three or five year limit of time are generally accepted, no uniform plan for the reporting of surgical statistics exists, so that it is practically impossible to compare the results from one clinic with those of another. This matter first attracted our attention in 1907, when we reported the end-results of operations for cancer of the breast at the Massachusetts General Hospital, and again in 1908, when we made a study of the end-results of operations of cancer of the mouth, tongue, and jaw. In the latter report the following statements were made:

> A comparison of these statistics with those of other writers was made and a large number of statistical papers were consulted. It was found, however, that the varying conditions under which the statistics were prepared made a comparison difficult without injustice to one or another writer. *It would seem that a generally accepted standard form for the report of end-results of operations for cancer should be secured.*
>
> In a recent paper on cancer of the breast an arbitrary standard was adopted, and that standard has been maintained in the present communication. It is, briefly, as follows:
>
> 1. A definite period of time has been selected ending at least three years prior to the report, and *all* of the cases entered in the hospital records under the given diagnosis have been investigated.
> 2. No case has been accepted as cancer without proof by pathological examination, or subsequent recurrence, or autopsy.
> 3. Cases which have survived, at last report, only a portion of the necessary three-year period, are eliminated as inconclusive.
> 4. Cases not traced at all after discharge from the hospital, and not appearing in the mortality statistics of their place of residence, are eliminated as inconclusive.
> 5. All cases fulfilling the above requirements are published and counted in the statistics with such subdivision into radical and palliative operations as may seem expedient.
>
> It is the hope of the writers that this standard requirement may be adopted by others for similar reports. A writer who counts as successful cases those which have survived a period of less than the arbitrary three-year standard, or who accepts the clinical diagnosis of cancer without microscopic examination of the specimen, cannot in justice ask to have his statistics accepted for comparison with those of surgeons who exercise a more careful scrutiny. It is well said by Halsted: "It is especially true of breast cancer that the surgeon interested in furnishing the best statistics may in perfectly honorable ways provide them."
>
> The obvious solution of this difficulty is to publish *all* cases which fulfill the present conditions and allow the reader to draw his own conclusions. If one surgeon reports 100 cases, of which 25 are palliative and 75 radical operations, his statistics for radical operations may well be expected to be inferior to those of the surgeon who performs 50 palliative and 50 radical operations in the same total number of cases. Without these figures, however, the opportunity for comparison is lost, and the two sets of statistics apparently meet on equal terms.

Since the method above described has proved satisfactory in operation, the writers would again present it as a standard suitable for adoption by the profession at large; and as a further example of its value they would here report, briefly, a second series of cases of cancer of the breast which were operated upon at the Massachusetts General Hospital between the dates of August 1, 1911, and April 1, 1914. During this period the policy of special assignments of cases to different members of the staff was in operation, and to the writers were given all cases of diseases of the breast which entered the hospital during that time. By following the same plan of reporting adopted in the earlier series a direct comparison of the work in the two series of cases is made possible. The only discrepancy lies in the fact that a five year period of time was adopted in the second series while the three year period was employed, as was customary at that time, in the earlier cases.

The scheme for the reporting may be summarized as follows:

A. Record all cases entering surgical wards with the specified diagnosis during period selected.
B. Eliminate all re-entries. (No single case should appear twice in the report).
C. Eliminate all cases recurrent after previous operation in hospital or elsewhere; these are not cases of primary attempt to cure.
D. Deducting $B+C$ from A we have the number of cases of cancer available for study of operability, mortality, and other operative statistics. These cases may then be subdivided as follows:
E. Cases of radical operation.
F. Cases of palliative operation.
G. No operation advised or performed.
H. Operative deaths.
I. Operative mortality $H \div E+F$.
J. Operability (radical operations) $E \div D$.
K. Operability (all operations) $E+F \div D$.
For the study of end-results of treatment certain cases included in D are of no value and should be deducted, viz:
L. Cases not proved to be cancer either by pathological examination of tissue, or recurrence, or autopsy.
M. Cases untraced after leaving hospital for required interval of time—three years, five years.
N. Cases that have died of other diseases within the required interval of time, and without evidence of recurrence.
O. The cases remaining after deducting L, M, and N, from D, are available for study of end-results, as follows:
P. Radical operations.
Q. Palliative operations.
R. No operation.
S. Number of cases alive without recurrence (3 years, 5 years).
T. Number of cases died (over 3 years or over 5 years) without recurrence.
U. Number of 3 year or 5 year "cures": all operations.
V. Number of 3 year or 5 year "cures": radical operations.
W. Percentage of 3 year or 5 year "cures": all operations $(U \div P+Q)$.
X. Percentage of 3 year or 5 year "cures": radical operations $(V \div P)$.

End-results: Carcinoma of the breast

	1894-1904	*1911-1914*
A. Total entries. Carcinoma Breast............	613	115
B. Re-entries (entered more than once)..........	80	8
C. Recurrence from previous operation...........	65	4

D. Cases available for study of operability, mortality, etc.	468	103
E. Radical operation	360	74
F. Palliative operation	56	20
G. No operation	52	9
H. Operative deaths	15	0
I. Operative mortality (H÷E+F)	3.6%	0
J. Operability: Radical operations (E÷D)	77%	72%
K. Operability: All operations (E+F÷D)	89%	91%
L. Inconclusive cases; Lack pathological examination		0
M. Inconclusive cases: Untraced	38	5
N. Inconclusive cases: Died within time limit	2	3
O. Cases available for end-result data	428	95
P. Radical operations	320	69
Q. Palliative operations	56	17
R. No operation	52	9
S. No. cases alive and well	64	22
T. No. cases died without recurrence	7	1
U. No. 5 year "cures" (all operations)	71	23
V. No. 5 year "cures" (radical operations)	67	22
W. Percentage of "cures" (all operations) (U÷P+Q)	19%	27%
X. Percentage of "cures" (radical operations) (V÷P)	21%	32%

This paper appears in full in the Boston Med. & Surg. Jour. 1921, clxxxv, 253.

DISCUSSION

Dr. Bell (Minneapolis): Very important considerations in the curability of carcinoma of the breast are the size of the tumor, regardless of its histologic type, and the presence of metastases in the axillary nodes at the time of the operation. Has Dr. Simmons any information on the cured cases relative to these points?

Dr. Simmons: We divided the cases clinically into four groups. In group 1 were placed cases having a small tumor and no palpable axillary nodes. Seventy-one per cent of these cases are living without recurrence. Group 2 comprised slightly more advanced cases with small palpable axillary nodes. Thirty-three per cent of the cases in this group are living. In group 3 were placed the advanced cases, with many axillary nodes, in which an attempt at cure by radical operation was made. Ten per cent of the cases in this group are living. Group 4 consisted of advanced cases in which a palliative operation only was attempted. Five per cent of this group, one case, are living.

There were two cases of colloid carcinoma in both of which there were axillary metastases. These cases are both living without recurrence.

Infection of the axillary nodes, as shown by the microscopic examination, had a distinct bearing on the prognosis of the case. Thus there were twenty-four per cent cures in the cases showing axillary infection, and fifty-six per cent cures in those where the nodes were not involved.

It is interesting to note that in sixty-nine per cent of the cases the radical operation did all that could be expected of it; that is, if the patient died she died from remote metastases and without recurrence in the scar or axilla.

10. Radium in Cancer of the Bladder

Dr. George Gilbert Smith (Boston):

SUMMARY

A report of experiences at the Huntington Memorial Hospital, Boston, with the use of radium in 24 cases of cancer of the bladder. Of these cases only 1 might have been suitable for radical removal. Seven cases were quoted to show the effect of single applications of radium in the effort to determine a standard dosage. Fifteen cases were treated by the introduction of screened radium emanation into the bladder cavity. Of these 9 cases had a total treatment of 600 mc. hours or more. Analysis of these cases showed that while 2 of them gave a temporary diminution in the amount of tumor and 1 possibly showed a total destruction of carcinoma, none was completely cured even clinically. Six cases were treated by the introduction of bare emanation tubes into the tumor itself. In 3 cases this was done without opening the bladder; in 3 it was done by cystotomy. Of these cases 1 appeared to be completely cured after 1 year. Another case dying six weeks after operation showed no carcinoma at autopsy. With the other cases insufficient time has elapsed to determine the result of the treatment.

Conclusions

1. It is useless to attempt to cure with radium infiltrating carcinomata, which involve large portions of the bladder wall. Necrosis of the bladder will be brought about by any dosage which will materially influence the tumor.
2. Certain superficial cancers of the bladder may be reduced in extent by the application of screened radium emanation to their surface. This may occur without necrosis of the bladder wall.
3. To accomplish this effect, 400 mc. hours, with screening of 0.5 mm. silver, applied not oftener than once in six weeks, has been successful, and has not caused any considerable reaction in the bladder.
4. The greatest effect is produced by the first 3 or 4 applications of radium.
5. If the tumor begins to grow again, further radium applications have little deterrent effect.
6. The best way to employ radium in cancer of the bladder is by the implantation of bare emanation tubes in the tumor, allowing one tube to each cubic centimeter. Steel needles containing radium may be employed in the same way, except that they must be withdrawn after adequate exposure has been made.

7. The necrosis caused by the implantation of radium in bladder tumors persists for at least three months.

DISCUSSION

Dr. Wood: I think that these papers by Dr. Smith and Dr. Simmons are admirable. It is important that we laboratory men check up our results on human beings; we care nothing for rats and mice in themselves. One interesting point is the statement that a radiated tumor is resistant after scar tissue has been formed. The observation has been made by others also. I can not think that the carcinoma cell undergoes any change, simply because of the presence of scar tissue. The radium dosage for cancer cells in tissues not highly vascular, or in contact with normal tissue, is very much higher than for the same cancer cells where thrombosis can take place and cause extensive destruction. For mouse cancer cells, the dose is 2400 mc. hours at a distance of two centimeters; you see how resistant the cell is. Now Dr. Simmons has shown that 630 mc. hours are all that can be allowed, so that we are still far from able to guarantee killing the cell, or from promising that other cells will in any way affect the life of the cancer cell. I do not believe, for example, that lymphocytes or connective tissue cells destroy cancer cells; there is not the slightest evidence to that effect. Indeed, the cancer cell may lie quiescent in the connective tissue for seven, ten, or fifteen years.

11. Problems in Cancer Research

Dr. Montrose Burrows (Saint Louis):

(Published in the Journal of Cancer Research, 1921, VI, 131)

DISCUSSION

Dr. Burrows: In reference to the discussion on the alkalinity of the blood in cancer patients, I wish to state that we have found no direct correlation between the alkalosis in many cancer patients and the anaemia which is invariably present. Alkalosis in anaemic patients is a well established fact. So far our conclusions in relation to anaemia have been drawn from a comparison of the blood tests for pH which we have had made and the blood picture recorded on the hospital history. We are now anxious to make all these tests for anaemia ourselves. We also feel sure that other conditions such as lesions of the pancreas and kidneys will alter materially the pH determinations of the blood in the cancer patients. Certain of our cases as well as others cited in the literature have indicated this fact.

In referring to Dr. Clowes's statement about a lipoid membrane, I wish to call his attention to the fact that such exists in the tissue culture. We noted this fact in the literature in an article on "The cultivation of bladder and prostatic tumors" (Jour. Urol., vol. 1, no. 1). In analyzing the property of stereotropism introduced for body cells by Harrison, we found that these cells were not in contact with the cover glass or free surface of the medium as Harrison stated, but separated from the surfaces by a surface film of substance which is insoluble in the medium. This was particularly easy to demonstrate in the cultures of bladder and prostatic carcinomata.

Returning to the question of blood alkalosis in cancer, our work has further suggested to us that the alkalosis is due to the addition of an alkaline substance from the tumor into the blood. The pH becomes changed because of the lack of a very active compensating mechanism for alkalies (Bayliss).

The demonstration of an alkalosis in the plant cancers and in the culture of the Bacillus tumefaciens gives further confirmation of this fact.

12. Massage and Metastasis

Dr. L. C. Knox (New York, by invitation): Presented by Dr. F. C. Wood:

SUMMARY

The general conditions underlying the formation of metastatic deposits from malignant tumors have long been of interest to the surgeon as determining the scope and direction of his operative procedures and even the feasibility of operation.

In this country, although the dermatologists have always made biopsies in doubtful cases, there has been during recent years a great deal of discussion among surgeons as to the danger of incising tumors. Isolated instances of apparent distribution following diagnostic incisions have led to the widespread feeling that such incisions tend to distribute the tumor. This attitude, strangely enough, has existed only in the United States; the English, French, and German surgeons do not seem to have considered the matter as of importance.

On the other hand, the relationship of massage to the production of tumors has excited but little interest in the minds of the practitioners of surgery and there are but few recorded clinical observations of the spreading of tumors by massage, though such distribution takes place very frequently under the administration of the mechanical treatment used by the osteopath and the chiropractor to "disperse" tumors. Some of the most extraordinary instances of widespread metastatic involvement of the entire body have been seen following massage of a carcinoma of the breast. Several instances, in which very extensive and early metastasis occurred after small tumors had been repeatedly examined and rather firmly squeezed or handled by the physician making the examination, called the writer's attention to the importance of this phase of the metastasis question.

Though the problem is one easily attacked from an experimental point of view, but little work has been done on it, with the exception of experiments by Dr. E. E. Tyzzer, who some years ago observed that massage of a mouse tumor considerably increased the number of metastases. His experiments were confined to only one type of tumor; and, therefore, it seemed of great practical and scientific interest to extend, if possible, his observations to a large variety of tumors of different histological forms so as to correlate the results obtained from animals with those observed in human beings.

It is obvious that a small-cell tumor in which the cells are closely related to the blood-vessels, such as the lymphosarcomata, would metastasize early and extensively, while the firmer fibrosarcomata might be expected to offer considerable resistance to the removal of tumor particles into the lymph-channels and the blood-vessels. As the lymphatic system in animals is not as extensive as that in man, metastasis is most frequent by way of the blood-vessels; hence, the tumor cells reach the lungs first, and the effect of massage would be expected to increase very greatly the number of secondary tumors in these organs. This was found to be the case.

Two series of experiments were carried out with the same technique, one two years after the other. The first consisted in the inoculation of mice and rats with six strains of carcinomata and four strains of sarcomata. About 500 animals survived for the completion of the experiment. As soon as the tumors were palpable, half of them were massaged for thirty seconds on alternate days for about two weeks; the other half were used for controls. After this, the tumors were removed by operation, and the animals were killed at the end of thirty days. The lungs of these animals, as well as those of the animals which died spontaneously during the course of the experiment, were examined microscopically, and the number of metastases and emboli was noted. In the second experiment only one strain, a polyhedral-cell sarcoma, was used with 50 mice. The results of the two experiments were approximately the same.

It was found that in all but three strains there was a larger percentage of lung emboli in the massaged mice than in the controls; that there was also usually a higher percentage of actively growing metastases; and that the total number of metastatic particles of both quiescent emboli and growing metastases constantly increased, the variation being from 1 per cent to 37 per cent. In this respect, little difference was found between the polyhedral-cell sarcomata and the carcinomata. It was found, also, that fibrosarcomata are not so apt to metastasize when manipulated as are the other types.

13. Further Experiments on Sensitization to Heat by Radiation

Dr. William T. Bovie (Boston):

SUMMARY

The experiments reported in this paper concern the changes which take place between the time of radiation and the appearance of the first visible effects.

At a previous meeting I have discussed the rate of recovery of Paramecium from the destructive action of fluorite rays, and the sensitization to heat resulting from the exposure to fluorite rays.

My interpretation of the results of these investigations was incorrect, because through faulty methods of experimentation the effects of ozone formed by the light were not excluded.

The experiments reported in the present communication were conducted in such a manner as to exclude the effects of ozone.

A very definite sensitization to heat was demonstrated.

By increasing the temperature of the organism after the radiation, the length of the latent period is shortened. All of the changes produced by the radiation are intensified and the total number of deaths for a constant dosage is increased.

The effect is greater the higher the temperature and the longer the time during which the organism is maintained at the increased temperature.

The temperatures used were not high enough to affect normal unradiated organisms, nor were the effects to be observed if the organisms were subjected to the increased temperature before the exposure to the rays.

DISCUSSION

Dr. Clowes: At what temperature do these changes start?

Dr. Bovie: Room temperature, 22°C.

Dr. Clowes: You must take into consideration variation in temperature, for radiation is ultimately chemical.

Dr. Bovie: We have a thermo-couple connected with the slide, and make certain that the temperature is back at normal before we radiate. It would not make a great difference, however, because light reactions are not influenced by changes in temperature.

Dr. Wood: It may interest Dr. Bovie to know that we are not able to detect any difference in the cancer cells between heating first and radiating afterward, or radiating first and heating afterward. The point we used was the death point determined by animal inoculation. This is far from what he is working with. We can observe it accurately. We get an approximation to a logarithmic curve but whether this is the true form of the curve is doubtful.

14. Malignant Tumors of the Thyroid

Dr. Louis B. Wilson (Rochester, Minn.):

SUMMARY

This paper presents an analysis of the pathological data concerning 290 patients with malignant tumors of the thyroid examined in the

Mayo Clinic between January 1, 1901, and January 1, 1921. Lantern slides illustrating the various histologic types of tumor were shown and the clinical course of the disease in cases of the various types discussed in connection therewith. The following is a summary of the principal points in the paper:

1. Malignant tumors of the thyroid are much more frequent than is generally believed. Correct clinical diagnosis is frequently missed, (a) because they may have periods of development of from five to fifteen years and patients are not followed up long enough after operation; and (b) because not infrequently the tumor is relatively small and the character of metastasis is not determined, owing to the rarity of necropsies.
2. Pathologic diagnosis is difficult owing to the great variation in the histology of the tumor and its resemblance to that of non-malignant processes.
3. There has been a marked failure of American surgeons to report their cases of malignant tumors of the thyroid; this should be corrected.
4. Sufficient observations are not at hand for determining the geographic incidence.
5. The age incidence at the date of diagnosis is greatest in the fifth decade.
6. The distribution by sex is about one man to two women.
7. Patients usually seek medical advice on the occasion of recent rapid growth in a long standing nodular tumor of the thyroid. Some give histories of slow continuous growth.
8. Early thorough operation gives a fair percentage of cures. Palliative operation in late cases with extensive local involvement is warranted.
9. Pathologic diagnosis must take into account the usual development of malignant tumors of the thyroid from proliferating embryonic adenomas.
10. The pathologist must be thoroughly familiar with the characteristics of proliferating adenomas (as first described by Langhans) in all their stages.
11. The pathologist must be on the lookout for a possible relationship between bizarre metastatic growths and tumors of the thyroid.
12. The pathologist, in his diagnosis for the guidance of the surgeon, must consider the relative preponderance of proliferative and degenerative processes in the tumor; but a proliferating adenoma in a patient of cancer age should not be considered benign unless the process of degeneration is very extensive and thoroughly overbalances that of proliferation.

DISCUSSION

Dr. Wells: My experience in the Chicago district corroborates Dr. Wilson's statements; there, also, tumors of the thyroid are not uncommon. It is hard to find a normal thyroid in experimental dogs in

Chicago, and I have many sent to me with carcinoma of the thyroid. There have been but a few definite tumors of the thyroid in mice in the Slye stock.

15. Experimental Production of Tumors

Dr. F. C. Wood:

SUMMARY

Dr. Wood reported the experimental production of tumors in the Crocker Laboratory by Dr. Frederick Bullock and Miss M. R. Curtis. The method employed was to feed rats with ova from cat feces. The ova were those of the Tenia crassicollis, a frequent inhabitant of the cat intestine.

The organism penetrates the mucous membrane of the intestine, passes to the liver and there forms a cyst. In the wall of such cysts, single or multiple sarcomata develop after a period which is never less than eight months. These sarcomata are of two main types, spindle-cell and large polyhedral-cell. They are highly malignant, metastasizing throughout the animals, and are transplantable, giving a high percentage of successful transplants at the first inoculation.

The importance of the discovery is the large yield of tumors and ease of the method, in contrast with the laborious tar painting process. One strain of animals gave approximately 50 per cent of tumors among all rats infected.

DISCUSSION

Dr. Bell: Has Dr. Wood tried any filtration experiments?

Dr. Wood: Some years ago large series of filtrations were carried out on various tumors in the Crocker Laboratory but without success.

Dr. Bell: I would like to ask whether the blood shows any changes in these very malignant sarcomata? Does it suggest a leukemia?

Dr. Wood: No changes at all.

FURTHER INVESTIGATIONS ON THE ORIGIN OF TUMORS IN MICE

VII. TUMOR AGE AND TUMOR INCIDENCE

LEO LOEB

From the Department of Comparative Pathology of Washington University School of Medicine, St. Louis

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1. In our former publications we have analysed the relation between tumor age and tumor rate in the various strains of mice (1). We found that in those strains in which the tumor rate was high the tumors tended to appear at an earlier period of life than in strains in which the tumor rate was lower. In addition, we found indications that there existed a special tumor age in certain strains of mice. In general cancer age was transmitted by heredity as well as cancer rate.

The present communication differs from our preceding ones in the following respects: (a) We analyse here our total material connectedly, while previously we considered only certain parts. (b) The method of computation of the relations between tumor age and tumor rate, which we used formerly, was complicated and made a comparison between different strains and groups of mice difficult. We now make use of a simpler way of figuring out the relation between tumor age and tumor rate and thus are enabled to compare more readily the different strains. (c) We extend our analysis in various directions and explain our results more fully on the basis of multiple factors.

2. In order to determine the tumor age of a certain family strain or group of strains we proceeded in the following manner. We determined the number of mice alive in the beginning of each of the three age periods (I age period, 7–12 months; II age period, 13–17 months; III age period, 18 months and older.) We

then determined the number of mice which developed tumors in each of these age periods and figured out what percentage of mice were in each age period affected by cancer. These percentage figures we then reduced to a basis of 100 per cent cancer incidence, in order to obtain figures for the cancer age in different strains which were independent of the absolute number of tumor mice in each strain. We may give as an example of this mode of determination the figures obtained for the total of all 8½ + 328 strains; the last figure on each line indicates the number of mice in which tumors developed in each age period in a unit of 100 tumor mice. The figure 56.4 per cent indicates the tumor incidence of the whole strain.

		8½ + 328		
I age period.	566 mice	194 tumor mice = 34%	27%	
II age period.	208 mice	117 tumor mice = 56%	44%	56.4%
III age period.	24 mice	9 tumor mice = 38%	29%	

3. We divide all our strains into high, medium, and low tumor rate strains. The high tumor rate strains have an incidence of more than 40 per cent. The medium tumor rate strains have an incidence varying between 20 and 40 per cent, and the low tumor rate strains have an incidence below 20 per cent.

In our lists we state first the name of the strain, then we give the number of mice and tumor rate in each strain and in the last three rows the tumor incidence in each age period, reduced to a unit of 100 tumor mice. Added in brackets to these figures are in certain strains the absolute percentage figures of tumors in each age period. Wherever the name of the strain as a whole is in brackets, the figures were merely given in the list; they were for obvious reasons not used in figuring out the incidence in each age period in all of the strains belonging to one of the classes (high, medium, low rate tumor mice).

If we compare the figures giving the percentage of mice affected by cancer in the three age periods, we may draw the following conclusions: (1) If the number of mice in a group is small, for instance, below 50, or even somewhat higher, the percentage age figures are no longer of value; if the number of mice exceeds one hundred, the percentage figures begin to be much more trust-

High rate tumor mice

STRAIN	NUMBER OF MICE	TUMOR RATE	PERCENTAGE OF TUMORS IN		
			I age period	II age period	III age period
		per cent	*per cent*	*per cent*	*per cent*
(London Blue and White).....	31	55.0	18.0 (26.0)	37.0 (54.0)	45.0 (66.0)
Total $8\frac{1}{2}$ + 328................	566	56.4	27.0 (34.0)	44.0 (56.0)	29.0 (38.0)
($8\frac{1}{2}$ + 328 A)..................	118	51.0	29.0 (34.0)	42.6 (50.0)	28.4 (33.3)
($8\frac{1}{2}$ + 328 B)..................	226	56.0	25.4 (32.0)	41.0 (52.5)	33.6 (42.0)
[$8\frac{1}{2}$ + 328 (new cross)].........	146	57.5	26.5	44.0	29.5
[782a (= $8\frac{1}{2}$ + 328)]............	76	60.0	35.0 (38.0)	65.0 (70.0)	0.0 (0)
(English)......................	24	46.0	25.0 (29.0)	32.0 (37.0)	43.0 (50.0)
(English A)....................	175	63.0	20.0 (29.0)	45.0 (64.0)	35.0 (50.0)
[Family 101 (English)].........	99	71.0	21.0 (45.5)	32.0 (69.5)	47.0 (100)
(English Sable A)..............	252	70.0	28.0 (46.0)	34.0 (54.5)	38.0 (62.0)
(English Sable B)..............	48	67.0	20.0 (27.0)	49.0 (65.0)	31.0 (40.0)
[Family 437 (English Sable)]...	55	82.0	37.0 (58.0)	63.0 (100)	0.0
(Smaller English Family).....	36	56.0	46.0 (42.0)	54.0 (50.0)	0.0
[344 + 328 and (344 + 328) + 437] English Sable..........	34	79.0	46.0	54.0	0.0
Total English..................	689	67.6	25.0 (40.6)	39.0 (62.5)	36.0 (54.5)
[(European 151 + Id of No. 10) + 101 (English) Total]......	62	42.0	25.0 (26.0)	36.0 (37.0)	39.0 (40.0)
[(European 151 + Id of No. 10) + 101 English A]............	27	55.0	40.0	60.0	0.0
" (English B)	35	31.0	17.7	24.0	58.3
Total Michigan Wild + English 101................	70	48.0	21.0 (23.0)	28.0 (31.0)	51.0 (57.0)
(English A)..................	50	58.0	19.0	29.0	52.0
(English B)..................	20	25.0	29.5	21.5	49.0
[European 151 + Id of No. 10 (November 3)]...............	254	72.0	15.0 (25.0)	33.0 (55.0)	52.0 (88.0)
Total European 151 + IId of No. 10 (November 8)	187	54.5	15.0	36.0	49.0
(Total European A).........	96	65.0	14.0	36.0	50.0
(Total European B).........	91	44.0	17.5	33.5	49.0
($8\frac{1}{2}$ + IId of No. 10) A.........	82	49.0	9.0	35.0	56.0
English Sable + [(European + 103) F_1 + IIId of No. 10] F_2.........................	6	83.0	0.0	38.0	62.0
European + English Tan (daughter of tumor mouse 146 B)......................	33	42.0	4.6	57.5	37.9
(Silver + 10 B)................	11	64.0	17.0	20.0	63.0
Total English Tan + German.	60	46.6	24.0	41.0	35.0
(English Tan + German A)...	44	49.0	21.0	35.0	44.0
(English Tan + German B)...	21	43.0	33.0	67.0	0.0

STRAIN	NUMBER OF MICE	TUMOR RATE	PERCENTAGE OF TUMORS IN		
			I age period	II age period	III age period
		per cent	*per cent*	*per cent*	*per cent*
Unknown (probably English)	32	72.0	30.0	36.0	34.0
Total 198 English + (8 + German)	217	54.0	20.0 (28.0)	41.0 (58.0)	39.0 (55.0)
[198 English + (8 + German A)]	82	63.0	31.0	69.0	0.0
[198 English + (8 + German B)]	135	49.0	18.5	35.0	46.5
(German A)	20	50.0	12.0	36.0	52.0
(8 + German A)	244	41.0	13.0	40.0	47.0
[Family 240 of (8 + German)]	14	43.0	29.0	33.0	0.0
Combined (Silver + English A) and Silver + (English Sable C)	40	57.5	23.0	57.0	20.0
English Sable 344 + German	45	64.5	20.5	26.0	53.5
English Sable + (English Silver + Id of No. 10)	23	65.0	23.0	77.0	0.0
English Sable + (European + 1d No. 10)	125	55.0	25.0	40.0	35.0
(English Sable + Cream Y)	68	53.0	14.0	46.0	40.0
693 (= English Tan + Cream) + 773 (= $8\frac{1}{2}$ + 328)	35	60.0	38.0	62.0	0.0
794 (German + Carter) + (8 + German)	90	49.0	18.0	25.0	57.0
794 (German + Carter) + [198 (English) + (No. 8 + German) F_3]	37	61.0	21.0	26.0	53.0
Waltzer + No. 8	52	46.0	20.5	31.5	48.0
240 (8 + German) + Cream	38	76.0	11.0	37.0	52.0
$8\frac{1}{2}$ + English Sable	48	61.0	40.6	59.0	0.0
Waltzer + English Orange and combined cross	65	65.0	36.0	64.0	0.0

worthy. (2) With this restriction as to numbers, we may conclude that the age distribution of tumors is at least as characteristic of strains and serviceable for their distinction as the tumor incidence; in fact in certain cases it is a finer instrument for the characterization of strains than the tumor rate. The tumor rate of two strains may be similar, but the tumor age may allow a differentiation between the two strains.

Thus if we consider especially the high tumor rate strains we notice that the tumor rate of European 151 and I daughter of No. 10 and of European 151 and II daughter of No. 10 on the one hand and of $8\frac{1}{2}$ + 328 and English and its substrains on the other hand do not differ very much, while the tumor age is quite distinct.

The percentage figures for the first and third age period are similar in the former two strains and differ very much from the figures in the latter strains. There is even a difference between English and $8\frac{1}{2}$ + 328, both very high rate tumor strains; in the English the tumors appear somewhat later than in the $8\frac{1}{2}$ + 328 strain. This comes out even more clearly if we consider the percentage figures for the third age period than those for the first age period.

4. If we compare the tumor ages of the substrains with those of the main strains we find that on the whole they agree provided the figures used are not too small. This applies for instance to the English, $8\frac{1}{2}$ + 328 and European + I or II daughter of No. 10 and their substrains.

In the case of the strain "unknown" there was some indication that it was a substrain of the English. Not only the tumor rate, but also the tumor age of this strain furnish confirmatory evidence for this conclusion.

5. In crosses between English and the majority of other strains in which the tumor incidence is high the tumor age is similar to that of the English. $8\frac{1}{2}$ + 328 is a cross of the female offspring of a particular English tumor mouse with an $8\frac{1}{2}$ male. Here we have, owing to the influence of the English mother, a very early tumor age which exceeded even the average age of the English mice. On the whole, we find the English or a similar tumor age in the following additional crosses: (European 151 + I daughter of No. 10) + 101 English, Michigan Wild + English 101. (European 151 + I daughter of No. 10) + 101 English) A. English Tan + German, 198 English + (8 + German), Silver + English, English Sable + German English Sable + (English Silver + I daughter of No. 10), English Sable + (European + I daughter of No. 10) (English Tan + Cream) + ($8\frac{1}{2}$ + 328),

Medium rate tumor mice

STRAIN	NUMBER OF MICE	TUMOR RATE	PERCENTAGE OF TUMORS IN		
			I age period	II age period	III age period
		per cent	*per cent*	*per cent*	*per cent*
(Complete English-Cream Hybrids)	1057	24.3	20.0 (8.6)	42.0 (18.0)	38.0 (16.1)
English-Cream Hybrids, medium rate	739	32.3	19.0 (11.3)	41.0 (24.4)	40.0 (24.0)
(English Sable 344 + Black Cream)	92	23.0	18.5	40.0	41.5
(Black Cream + English White)	143	39.0	19.0	47.0	34.0
(Cream + English, October, 1913)	109	23.0	12.5	31.0	56.5
(White Cream + White English)	27	29.0	36.0	26.0	38.0
[Cream-English (Descendants of English Sable 1031)]	26	31.0	6.9	44.6	48.5
(English Sable + Cream Y, high rate)	68	53.0	14.0	46.0	40.0
(693 + Cream)	7	28.5			
[Total English Tan (121) + Cream]	267	31.0	22.0 (12.0)	38.0 (21.0)	40.0 (22.0)
Total London	452	28.0	22.0 (10.9)	45.0 (22.0)	33.0 (16.0)
(London A)	120	27.0	13.0 (7.5)	36.0 (19.0)	51.0 (27.0)
(London B)	61	38.0	29.0 (16.0)	56.5 (32.0)	15.8 (8.0)
(London C)	197	28.0	11.0 (25.5)	49.0 (21.0)	25.5 (11.0)
(London 481)	43	0.0			
(London Blue and White)	31	55.0	18.0	37.0	45.0
Total European + 8 F_5	151	28.0	12.5 (6.0)	44.25 (21)	44.25 (21)
(European + 8 F_5A)	125	30.0	12.5	48.0	39.5
(European + 8 F_5B)	26	23.0	9.5	26.0	64.5
Total Heitler	196	25.0	12.5 (61.0)	64.0 (31.0)	23.5 (11.5)
(Heitler A)	102	27.0	8.5 (4.0)	91.5 (43.0)	0.0 (0.0)
(Heitler B)	94	22.3	19.0 (8.5)	39.0 (18.0)	42.0 (19.0)
101 (English) + (European + 103)	152	34.0	5.5 (4.0)	39.5 (29.0)	55.0 (40.0)
Total European + English Tan	109	32.1	7.4 (4.0)	50.0 (27.0)	42.6 (23.0)
(European + English Tan A)	76	28.0	4.0	45.0	51.0
(European + English Tan B)	33	42.0	4.6	57.5	37.9
(June, 1914, Family of Cream B)	40	35.0	4.6	33.5	61.9
Total No. 8	243	27.5	12.0 (7.0)	27.5 (16.0)	60.6 (35.0)
(No. 8 A)	213	30.0	11.4 (7.1)	32.2 (19.0)	56.4 (36.0)
No. 8 A_1	145	34.0	17.0 (11.0)	33.0 (21.5)	50.0 (32.0)
(No. 8 A_2)	68	22.0	0.0 (0.0)	24.5 (15.4)	75.5 (47.3)
(No. 8 B)	30	10.0	0.0 (0.0)	0.0 (0.0)	100 (20.0)
Carter	67	39.0	26.0	32.0	42.0

STRAIN	NUMBER OF MICE	TUMOR RATE	PERCENTAGE OF TUMORS IN		
			I age period	II age period	III age period
		per cent	*per cent*	*per cent*	*per cent*
Total 8½ + II daughter of No. 10	110	40.0	8.5	40.0	51.5
(8½ + II daughter of No. 10 A)	82	49.0	9.0	35.0	56.0
(8½ + II daughter of No. 10 B)	28	14.0	0.0	100	0.0
(8½ + II daughter of No. 10) + IId of No. 10	72	36.0	8.9	20.8	70.3
(European 151 + Id of No. 10) + 101 English B	35	31.0	17.7	24.0	58.3
Total Silver + Id of No. 10	245	37.0	17.0	27.0	56.0
(Silver + 10 A)	234	36.0	17.0	26.0	57.0
(Silver + 10 B)	11	64.0	17.0	20.0	63.0
Total medium rate Cream + Id of No. 10	370	34.0	10.0 (5.4)	32.0 (17.0)	58.0 (31.0)
(Total Cream + 10 includes one low rate cross)	498	27.5	8.4	29.0	62.6
(Cream + 10 A)	174	36.0	5.5	28.0	66.5
(Cream + 10 B)	47	26.0	22.0	46.0	32.0
(White Cream + Id of No. 10)	149	34.0	12.0 (8.0)	33.0 (20.0)	55.0 (38.0)
(Michigan Wild + English 101 B)	20	25.0	29.5	21.5	49.0
Total German	42	40.5	15.5 (19.0)	30.3 (37.0)	54.2 (66.0)
(German A)	20	50.0	12.0	36.0	52.0
(German B)	22	32.0	23.0	51.0	0.0
Total 8 + German	373	34.0	14.0 (8.0)	42.0 (24.0)	44.0 (25.0)
(8 + German A)	244	41.0	13.0	40.0	47.0
(8 + German B)	129	21.0	23.0	51.0	26.0
(Family 240 = 8 + German)	14	43.0	47.0	53.0	0.0
German + Carter B	113	33.5	22.0	31.0	47.0
794 =. German + Carter	25	36.0	5.0	29.0	66.0
English (344) + 8½ F_4 and F_6	155	25.0	21.0	39.5	39.5
Cream + [198 (English) + (8 + German) F_4]	33	33.5	36.5 (15.0)	73.5 (26.0)	0.0 (0.0)
Black Cream + European	104	32.0	11.0	32.0	57.0
European + Cream	25	28.0	5.3	49.0	65.7
(Waltzer + White English) + (Cream + 10)	19	21.0	18.9	81.1	0.0
Vermont Wild + English F_3	373	36.3	11.0	30.0	59.0
(⅞ Waltzer ⅛ Cream) + No. 8	32	31.0	28.6	46.4	25.0
German + No. 6 F_4	52	37.0	11.9	24.0	64.1
Waltzer + White English and (Waltzer + White English) + English	99	26.0	29.0	28.0	43.0
(Cream B June 1914 Family)	40	35.0	4.6	33.5	61.9

German + Carter + (198 (English) + (No. 8 + German) F_3), 8½ + English Sable, Waltzer + English Orange. Waltzer No. 8 is similar. It is different, however, in a cross between English and Cream which approached the tumor rate of the English, namely, English Sable + Cream Y. The tumor rate is here 55 per cent, but the tumor age is distinctly different; it is intermediate between that of the English and Cream.

German, 8 + German, German + Carter have a later tumor age; the same applies to the crosses between these strains. Somewhat later even is the tumor age of (8 + German) + Cream, owing to the influence of the Cream; yet the influence of the 8 + German causes the tumor age to be earlier than that of the Cream. In a similar way the tumor age of the (8½ + II daughter of No. 10) A is later, although in this group the tumor rate is high.

MEDIUM RATE TUMOR MICE

6. While in the large majority of the hybrids between Cream and English the tumor rate is intermediate (32.3 per cent) between that of the Cream and English strain, in some of them it is low like that of the Cream, and in one of them, as we have seen, it approaches that of the English strain. We have divided these hybrids in two larger groups, one containing the low and the other the medium tumor rate strains. We find that the tumor age in both reaches almost, but not quite, that of the English parent. Tumors appear relatively early in these groups; only in two of these hybrid strains the tumor age is intermediate. Again we notice that in small strains comprising only 26 or 27 individuals the determination of the tumor age is no longer certain.

7. The result as to the effect of hybridization on tumor age is different in another hybrid strain in which Cream was crossed with the No. 10 (I daughter) strain. While in this case the tumor rate is intermediate, the tumor age approaches that of the Cream, although it does not quite reach the lateness of the tumor age of the Cream. On the other hand, in a cross between Silver and the same No. 10 strain, although the tumor rate is

again intermediate—in accordance with the low tumor rate of Silver which almost corresponds to that of the Cream—the tumor age is decidedly different and very much resembles that of the No. 10 strain; this is in accordance with the fact that, as far as can be determined from the restricted number of Silver mice at our disposal, the tumor age is in this latter strain very much higher than that of the Cream.

This dissociation between tumor age and tumor rate is likewise apparent in the London strain, where the tumor age almost reaches that of the English, while the tumor rate is very much lower.

A similar dissociation we find in the following strains: Carter (German + Carter) B, English (344) + 8½, Cream + (198 English + (8 + German) F_4) and in the Waltzer and English as well as the Waltzer and Cream hybrids. In the case of the Cream + (198 English + (8 + German) F_4) strain the number of mice is perhaps too small to permit much consideration. The Carter strain is in its origin related to the English; the tumor rate in this case stands on the border between a medium and high rate. The other strains in this class are hybrids in which either English, Carter, or Waltzer mice enter. In these hybrids there seems to be a tendency to an early tumor age. This would agree with the early tumor age of the English and Carter parent strains. In regard to the pure Waltzers, we have no statistics as to their tumor age and tumor rate. To judge from the result in the crosses we can only infer that their tumor age was early.

8. In a number of tumor strains with medium tumor rate the tumor age corresponds to the rate. (11 to 15 per cent of tumor mice in the I age period, an average of 45 to 55 per cent in the III age period.) In this group we include: European + No. 8, Heitler, German, 8 + German, No. 8, White Cream + I daughter of No. 10, Black Cream + European, Vermont Wild + English F_3 and German + No. 6 F_4. Of these, Black Cream + European and Vermont Wild + English stand on the border between a medium and late tumor age.

9. There are some strains with a medium tumor rate and a late tumor age. To this group belong: 101 (English) + (Euro-

pean + 103), European + English Tan, 8½ + II daughter of No. 10, (8½ + II daughter of No. 10) + IId. of No. 10, Cream + I daughter of No. 10, and European + Cream. We notice that the parents (European + 103), European 8½, and Cream have the tendency to impart to hybrid strains a late tumor age. All those parent strains have either themselves a late tumor age or their tumor age is on the border between medium and late, with exception of the European which, notwithstanding their relatively early tumors, likewise seem to impart a late tumor age to their hybrid offspring. In this strain there was perhaps present a greater individual variability in the tendency towards a certain tumor age. We notice furthermore that whenever the I or II daughter of No. 10 enters into a cross with a second parent strain with a tumor age which is late or approaches lateness, the No. 10 component of the cross, despite its high tumor rate, does not tend to make the tumor age earlier, because the European + I and II daughter of the No. 10 mice themselves have a relatively late tumor age considering their high tumor rate.

10. If we compare the list of the high tumor rate strains with that of the medium tumor rate strains, we find in the former the tumor age on the whole much more homogeneous than in the latter. In the high tumor rate strains the tumor age in all the important strains with exception of strains into which the daughters of No. 10 enter is early, while in the medium tumor rate strains the variability as to tumor age is much greater, although the deviations are about equally distributed on both sides of a medium tumor age, which latter represents the average.

LOW STRAIN TUMOR MICE

11. The Cream strain is typical of the low rate tumor strains. The difference in the tumor age between this strain and a typical high rate strain is striking; the tumors appear here much later in life than in the high tumor rate strain. We must of course take into consideration the fact that the smaller the number of tumor mice, the greater is the effect on the percentage age distribution of a small variation in the number of mice dying in the different age periods. Therefore we should expect a

Low rate tumor mice

STRAIN	NUMBER OF MICE	TUMOR RATE	PERCENTAGE OF TUMORS IN		
			I age period	II age period	III age period
		per cent	*per cent*	*per cent*	*per cent*
Total English Cream low rate hybrids	318	5.7	28.0 (2.5)	38.0 (3.4)	34.0 (3.0)
(344 + Black Cream) F_1 + Cream (white)	11	0.0	0.0	0.0	0.0
(English 344 + Cream, new individuals)	143	9.0	23.4	37.0	39.6
(English Sable 4444 + Cream)	175	2.8	30.0	36.0	34.0
Total Cream	878	5.9	8.0 (1.0)	26.0 (3.3)	66.0 (8.2)
(Total Cream ×)	260	2.7	18.2 (0.8)	52.3 (2.3)	29.5 (1.3)
(Cream × A)	135	4.0	27.0 (1.4)	73.0 (3.8)	0.0 (0.0)
(Cream × B)	77	2.6	0.0 (0.0)	33.0 (2.0)	67.0 (4.0)
(Cream × C)	48	0.0	0.0	0.0	0.0
(Old Cream)	226	2.0	0.0 (0.0)	20.0 (1.2)	80.0 (4.3)
(Cream A, mostly black)	114	8.0	10.4 (1.7)	23.0 (3.7)	67.0 (11.0)
(Cream B, mostly white color)	89	19.0	3.0 (1.1)	39.0 (14.0)	58.0 (21.0)
(Among Cream B, June 1914 Family)	40	35.0	4.6 (2.5)	33.5 (18.0)	61.9 (33.0)
(The rest of Cream B after deduction of June 1914 family)	49	6.0			
(Cream Black II)	77	4.0	13.0 (1.3)	0.0 (0.0)	87.0 (8.5)
(Cream Black III)	98	11.0	13.6 (3.0)	14.0 (3.1)	72.4 (16.0)
(Cream Y)	14	0.0	0.0	0.0	0.0
(Total new Cream)	349	10.5	7.6 (1.7)	25.0 (5.6)	67.4 (15.0)
Total European	263	9.0	21.0 (3.4)	34.0 (5.4)	45.0 (7.4)
(European A)	113	16.0	21.0 (6.0)	42.0 (12.0)	37.0 (11.0)
(European B)	150	3.3	18.0 (1.3)	14.0 (1.0)	68.0 (5.0)
Total European + 102 or 103	221	15.0	3.0 (1.0)	24.0 (7.5)	73.0 (23.0)
(European + 102 or 103 A)	146	20.5	4.3 (1.5)	23.0 (8.0)	71.7 (25.0)
(European + 102 or 103 B)	75	5.3	0.0 (0.0)	30.5 (5.5)	69.5 (12.5)
(London 481)	43	0.0	0.0	0.0	0.0
Total London + (European + 103) F_3	148	7.0	6.0	25.0	69.0
[London + (European + 103) F_3 A]	85	5.0	13.0 (1.2)	30.0 (2.8)	57.0 (5.4)
[London + (European + 103) F_3 B]	63	8.0	0.0 (0.0)	31.0 (4.1)	69.0 (9.0)
415 = [101 English + (European + 103)]	80	12.5	12.0 (3.5)	41.0 (12.0)	47.0 (3.5)
Total No. 8½	241	13.0	11.0 (2.9)	50.0 (16.0)	39.0 (10.0)
(No. 8½ A)	158	17.0	13.3 (4.0)	53.0 (16.0)	33.7 (10.0)
(No. 8½ B)	28	0.0	0.0	0.0	0.0

STRAIN	NUMBER OF MICE	TUMOR RATE	PERCENTAGE OF TUMORS IN		
			I age period	II age period	III age period
		per cent	per cent	per cent	per cent
(No. 8½ C)	55	9.0	7.5 (2.0)	33.0 (9.0)	59.5 (16.0)
Total (European + 102) F_1 + 8½ F_4	602	16.0	6.5	21.0	72.5
[(European + 102) F_1 + 8½ F_4 A]	473	16.0	7.0	18.0	75.0
[(European + 102) F_1 + 8½ F_4 B]	129	15.0	8.6	28.5	62.9
Total (European + 103) F_1 + IIId of No. 10	219	14.6	4.4	29.0	66.6
[(European + 103) F_1 + IIId of No. 10 A]	168	17.0	6.3	28.0	65.7
[(European + 103) F_1 + IIId of No. 10 B]	30	13.0	0.0	10.7	89.3
[(European + 103) F_1 + IIId of No. 10 C]	21	0.0	0.0	0.0	0.0
English Silver	127	7.0	35.0 (3.5)	65.0 (6.6)	0.0 (0.0)
English Silver Fawn	57	12.0	0.0 (0.0)	38.0 (11.0)	62.0 (18.0)
London + Silver	176	12.0	8.0	24.0	68.0
(No. 8 B)	30	10.0	0.0	0.0	100
(8½ + IId of No. 10 B)	28	14.0	0.0	100	0.0
German + Carter A	358	9.0	6.6	26.0	67.4
English Sable 344 + (European + English 146) (344–146)	21	5.0	0.0	100	0.0
White Cream + (European 151 + Id of No. 10 = No. 697)	128	9.5	5.8 (1.65)	17.5 (5.0)	76.7 (22.0)
Cream + (Cream + European 428) F_1	11	10.0	0.0	100	0.0
German + No. 8	112	0.0	0.0	0.0	0.0

much greater variability in the age distribution in the low rate than in high rate tumor strains. This does not, however, apply in the case of the representative strains of this group because we are working with a very great number of individuals in these strains. Thus, while in the Cream strain the tumor rate is as low as 5.9 per cent, the strain consists of 878 mice and among them are 52 tumor mice, a sufficiently large number for our purpose. If we consider, on the other hand, the substrains in this group, then the number of tumor mice usually becomes so small that the development of one or two more tumor mice in an earlier age period may change considerably the age distribution. Notwithstanding this fact we find in the Cream strain that the smaller substrains behave essentially like the main strain; we notice a low percentage of tumor mice in the first age period and a high percentage in the third age period, with exception of the Cream X A substrain where we have to deal with only 5 tumor mice, two of which died in the first and three in the third age period, and even here a change in time of death in the case of one or two individuals would have made the age curve typical.

12. What applies in the case of the Cream strain applies also to the other more important low rate tumor strains, as for instance the European + 102 or 103 where, as a result of the somewhat higher tumor rate, we have to deal with 34 tumor mice. It also applies in the case of London + (European + 103) F_3, in the large strain (European + 102) F_1 + $8\frac{1}{2}$ F_4, where we can base our calculations on 97 tumor mice; furthermore, in the case of the (European + 103) + III daughter of No. 10, London + Silver, German + Carter A in which the tumor rate was only 9 per cent and the age distribution was accordingly typical for the low rate tumor strain, while in the German + Carter B, where the tumor rate rose, the percentage distribution in the different age periods also changed in the typical manner.

The same age distribution we find furthermore in the White Cream + (European 151 + I daughter of No. 10) and some smaller strains. In the German + 8 tumors did not develop. Wherever the European + 102 or 103 enter a cross, they not only depress the tumor rate, but call forth the typical age distribution characteristic of the low tumor rate strains. This applies even in the case of the English 101 + (European + 103) where the association with the English strain succeeded in raising the tumor rate to 34 per cent and thus in producing a medium rate tumor strain; but the tumor age is very nearly as late as that of the Cream.

13. There are a few groups in this class in which the tumors appear earlier, namely, European, a small substrain 415 = 101 English + (European + 103), No. $8\frac{1}{2}$, and English silver. Eng-

lish Silver is related to the English and the tumor age approaches that of the English. Notwithstanding the relatively small number of tumor mice in this strain, this age distribution is probably not altogether due to a coincidence; at least we may interpret in this sense the fact that even in the hybrids in which English Silver enters the tumor age is relatively early. In No. 8½ and in 415 = 101 + (European + 103) the tumor age stands at the border between the early and medium types, or rather it approaches the medium type. In the European strain the tumors appear earlier. Here we may have to deal with another instance in which tumor rate and tumor age are dissociated; in the case of the low rate tumor mice the number of tumor mice is, however, relatively so small that in individual strains, except the largest ones, chance variations cannot be excluded with certainty.

14. In the English-Cream hybrid with a low tumor rate the tumor age is very early, similar to that of the English. The number of mice is 318, the tumor rate is 5.7 per cent; we have to deal with 18 tumor mice. This corresponds to the similar high tumor age in the English-Cream hybrids with medium tumor rate. In this group the number of mice is still greater and our classification is based on the cancer age of 239 tumor mice.

We may therefore conclude that in the English-Cream hybrids, independently of the tumor rate which may vary in different cases, the tumor age approaches that of the English. This applies even in the case of the two individual English-Cream hybrids with a low tumor rate.

In the case of the English-Cream hybrids we find, therefore, a splitting in the inheritance of tumor rate and tumor age.

THE TUMOR AGE OF THE HIGH, AND LOW TUMOR RATE MEDIUM STRAINS

15. The great difference in the tumor age of the high, medium, and low tumor rate strains is brought out very clearly if we distribute all our strains among three great groups, according as to

whether their tumor rate is high, medium, or low, treating each group as a whole and determining in each of them the tumor age of the group.

Thus we obtain the following figures:

Total of the high tumor rate strains

I age period.	2741 mice	885 tumors = 32.3%	22.3%
II age period.	1176 mice	636 tumors = 54.1%	37.3%
III age period.	225 mice	132 tumors = 58.7%	40.4%
		Average tumor rate	60.3%

Total of the medium tumor rate strains

I age period.	4351 mice	394 tumors = 9.0%	14.6%
II age period.	2855 mice	635 tumors = 22.2%	36.0%
III age period.	1238 mice	374 tumors = 30.5%	49.4%
		Average tumor rate	32.2%

Total of the low tumor rate strains

I age period.	3971 mice	67 tumors = 1.69%	8.5%
II age period.	2747 mice	152 tumors = 5.53%	27.8%
III age period.	1362 mice	173 tumors = 12.7%	63.7%
		Average tumor rate	9.9%

This arrangement brings out very clearly the difference in the tumor age of the different groups. In the group composed of the high tumor rate strains the average tumor rate is 60.3 per cent, the tumors appear early, almost, but not quite, as early as in the English strain. The figures for the I, II, and III age period are I 22.3 per cent, II 37.3 per cent, III 40.4 per cent. In the second group the average tumor rate is 32.3 per cent. The tumor age is correspondingly intermediate. The figures for the 3 age periods are: I 14.6 per cent, II 36 per cent, III 49.4 per cent. In the third group the average tumor rate is 9.9 per cent. The average tumor age is approximately that of the Cream; the figures are I 8.5 per cent, II 27.8 per cent, III 63.7 per cent. We may therefore conclude that the higher the tumor rate in a strain, the earlier the tumors appear on the average; the greater is the number of tumors in the first age period, and the smaller is the number in the third age period. The differences in the first age period are, however, somewhat greater than those in the third age period. The differences between the figures for the II age period, which represent the turning point are, as might be expected, much smaller in the three groups.

16. This relationship between tumor rate and tumor age can be shown in still another way. We may arrange all the strains in three different classes in accordance with their tumor age, the first class comprising those strains having a tumor age similar to that of the high tumor rate group, the second comprising those strains having a tumor age similar to that of the medium tumor rate group, and the third comprising the strains with a tumor age similar to that of the low tumor rate group.

We then find in the first class, comprising the strains with an early tumor age, 19 strains with a high tumor rate, 10 strains with a medium tumor rate, and 3 strains with a low tumor rate. Almost 60 per cent of the strains belong, therefore, to the high tumor rate strains.

In the second class, with a medium tumor age, we find 10 strains with a medium tumor rate, 2 strains with a low tumor rate, and 5 strains with a high tumor rate. In this class 59 per cent of the strains belong to the medium tumor rate strains.

In the third class, with a late tumor age, we find 14 strains with a low tumor rate, 6 strains with a medium tumor rate, and 1 strain with a high tumor rate. Sixty-seven per cent of the strains belong to the low tumor rate strains. In each class the majority of strains have a tumor rate which corresponds to the tumor age of that class.

ON THE PERIOD OF LIFE IN WHICH THE DEATH-RATE FROM CANCER IS HIGHEST IN MICE

17. It is generally assumed that in man the cancer incidence on the whole increases with increasing age. However, we find certain variations according to the kind of cancer which is considered; thus the death-rate from mammary and uterine cancer reaches a somewhat earlier maximum than the death-rate from cancer in general. The same applies to sarcoma. In mice Murray finds the death-rate from mammary cancer to reach a maximum at the age of thirteen to sixteen months. Murray's

statistics differ from our own in that they are based on a much smaller number of mice and in the method of computation he uses. Later we shall apply the same method also to our material.

We found that the age of death from mammary cancer in mice is not a fixed point, but varies with the cancer rate. The cancer rate being variable in different strains, the cancer age is likewise variable. In general we may state that the cancer age is the earlier, the higher the cancer incidence.

18. If we consider the total figures for high, medium, and low tumor rate strains respectively, we find that in the high tumor rate strains the difference between the cancer rates in the third and second age periods is very small, but the cancer rate is slightly higher in the third age period. In mice above the age of eighteen months the cancer rate is therefore slightly higher than in mice between the age of twelve and eighteen months. The maximum still is in the third age period.

In the medium tumor rate strains the difference between the cancer rate in the second and third age periods is more marked; the maximum is here quite definitely in the third age period. In the low tumor rate strains the difference between the tumor rate in the second and third age periods is still much greater; the maximum is very decidedly in the third age period. The lower the tumor rate, the more decidedly the maximum moves to the third age period. The lower the tumor incidence, the greater the maximum reached in the third age period and the greater the difference between the tumor incidence in the second and third age period. The difference between the tumor rates in the first and second age periods varies less; it happens to be least in the group of the high rate tumor strains.

19. If instead of considering the groups as a whole, we consider the individual strains composing them, the difference in the situation of the maximum tumor incidence in different strains comes out still more clearly. Thus in the 8½ + 328, a high tumor rate strain, the maximum is decidedly in the second age period, while in the English, although they have a slightly higher tumor rate, the maximum is somewhat higher than in the 8½ + 328, although it is still in the second age period if we consider the

total strain; the difference between the second and third age periods is however very small in this case and in the substrains we find some variation as to the maximum.

The great majority of the English hybrid strains behave like the English strain as far as the maximum of the tumor incidence is concerned. Similarly to the English strain behave in this respect particularly the English-Cream hybrids and European + English Tan, irrespective of their tumor rate.

In addition to various English hybrids, the strains London, 8 + German, and 8½ show a maximum similar to that of the English mice. On the other hand, in the European 151 + I or II daughter of No. 10, in which the tumor incidence is very high, the maximum is decidedly in the third age period.

We must then conclude that the maximum of tumor incidence is found at a different age level in different strains, and that this peculiarity is transmitted by heredity in the same way as the other characteristics of the strain.

The data as to the age period in which the maximum cancer incidence is found in mice, which we present here, permit only comparative conclusions. We are at present operating with three age periods which are not equal in length and we cannot therefore state at which age period the maximum occurs. But our data have a relative value. They show the shifting of the age period in different strains in dependence upon the variations in the cancer rate of the various strains and groups. And we may furthermore conclude that in all statistics so far no account has been taken of this variability of the tumor age in general and of the maximum in the tumor rate in particular. All available statistics give an average, in which this variable factor is disregarded and in which necessarily the result will differ in accordance with the relative preponderance of high or low tumor rate families or strains.

20. If instead of considering the three groups of mice separately we determine the tumor incidence in the three age periods in all the mice, irrespective of the group to which they belong, we obtain the following figures: In the first age period there were 11,063 mice; 1346 mice developed tumors during this period = 12.2 per cent.

In the second age period there were 6778 mice with 1423 tumor mice = 21 per cent.

In the third age period there were altogether 2825 mice, with 679 tumor mice = 24 per cent.

If we calculate these figures on the basis of a tumor incidence of 100 per cent, we find for the first age period a tumor incidence of 21 per cent, for the second age period an incidence of 36.7 per cent, and for the third age period an incidence of 42.3 per cent. These figures are somewhat between those of the high and medium tumor rate mice, but they approach very closely those of the high tumor rate mice. The maximum of the tumor incidence is accordingly in the third age class, but the difference between the figures for the second and third age period is relatively small.

21. If instead of considering in each age period the number of mice alive at the beginning of this period and the number of mice developing tumors in this period, and on this basis determining the percentage figures of tumor incidence for each age period, we determine what percentage of mice will develop cancer during the whole period following this date irrespective of the number of mice eliminated through death during each period, we obtain a different result. We had altogether 11063 mice which reached the age above six months. Of these 3448 subsequently developed cancer = 31.2 per cent; of 6778 mice which reached the age of twelve months, 2102 later developed cancer = 31 per cent; and of 2825 mice reaching the age of eighteen months, 679 developed cancer = 24 per cent. Figured out on this basis the tumor rate is similar for mice reaching the age of six and of twelve months. In these groups the tumor rate is higher than in mice reaching the age of eighteen months.

This mode of computation seems to us less satisfactory than the first method used, because it includes the number of individuals dying in each period among those which are liable to be affected by cancer in subsequent periods, and it thus makes the tumor incidence in the first two age periods too high.

22. If we leave out of consideration the mice dying below the age of 6 months we find among our material altogether a tumor incidence of 31.2 per cent. This is a relatively high tumor rate

which presumably exceeds the average tumor rate among white mice in unselected strains. Our material is a selected one, in which a special value was attached to high tumor rate strains.

23. If we determine with the second method the cancer incidence in the various groups of high, medium, and low tumor rate mice, we find the following figures.

High tumor rate mice

I 2741 mice 1653 tumor mice = 60.3%
II 1176 mice 768 tumor mice = 65.3%
III 225 mice 132 tumor mice = 58.7%

Medium tumor rate mice

I 4351 mice 1403 tumor mice = 32.3%
II 2855 mice 1009 tumor mice = 35.3%
III 1238 mice 374 tumor mice = 30.2%

Low tumor rate mice

I 3971 mice 392 tumor mice = 9.9%
II 2747 mice 325 tumor mice = 11.7%
III 1362 mice 173 tumor mice = 12.7%

On this basis the percentage figures for the different age periods are very similar in the three groups. But in the high and medium tumor rate mice the maximum of the tumor incidence is in the second age period, in the low tumor rate mice it is again in the third age period. Even this method, which is more faulty than the first one, brings out the shifting in the tumor incidence with varying tumor rates.

THE INHERITANCE OF TUMOR AGE AND INCIDENCE IN HYBRIDS

24. We have already referred to the behavior of individual hybrid strains as far as the relation between tumor age and tumor rate is concerned. We have also referred to some of the larger groups of hybrids. We shall now discuss the behavior of hybrids in some of the larger groups connectedly and compare their tumor rate and age with that of the parent strains.

(*a*). *English-Cream hybrids.* The figures for the parents are as follows:

A. Total English

I	689 mice	280 tumor mice = 40.6%	25%	
II	258 mice	161 tumor mice = 62.5%	39%	67.6%
III	46 mice	25 tumor mice = 54.5%	36%	

B. Total Cream

I	878 mice	9 tumor mice = 1.0%	8%	
II	600 mice	20 tumor mice = 3.3%	26%	5.9%
III	279 mice	23 tumor mice = 8.2%	66%	

C. Total English-Cream Hybrids

I	1057 mice	92 tumor mice = 8.6%	20%	
II	668 mice	120 tumor mice = 18.0%	42%	24.3%
III	280 mice	45 tumor mice = 16.1%	38%	

In these hybrids the tumor rate is on the whole intermediate between those of the parents, although somewhat nearer the Cream parent than the English. The tumor age, on the other hand, is almost that of the English parent. In this case we have to deal with such large figures that we are justified in assuming that these figures express causal relations and are not due to accidental findings.

The same relation comes out still more clearly if we divide the English-Cream hybrids into three subgroups.

A. Total English-Cream Hybrids with a medium tumor rate

(and with a slight admixture of high rate tumor mice)

I	434 mice	53 tumor mice = 12.0%	20%	
II	264 mice	67 tumor mice = 25.0%	41%	32%
III	100 mice	21 tumor mice = 23.0%	39%	

B. Total English-Cream Hybrids with a low tumor rate

I	318 mice	8 tumor mice = 2.5%	28%	
II	205 mice	7 tumor mice = 3.4%	38%	5.7%
III	101 mice	3 tumor mice = 3.0%	34%	

In this case the tumor rate is altogether that of the Cream, but again the tumor age is that of the English parent strain.

C. Total English Tan (121) + *Cream*

I	267 mice	32 tumor mice = 12%	22%	
II	173 mice	36 tumor mice = 21%	38%	31%
III	63 mice	14 tumor mice = 22%	40%	

Also in this strain of hybrids the tumor rate is intermediate while the tumor age is that of the English parent strain.

b. The same conclusion, that factors for tumor rate and age may be transmitted independently of one another, follows from a consideration of the hybrids between Cream and European 151 + I daughter of No. 10.

The tumor rate and tumor age of the Cream we have already given.

Figures for the mother strain European 151 + I daughter of No. 10 are as follows:

I	254 mice	63 tumor mice = 25.0%	15.0%	
II	148 mice	120 tumor mice = 81.0%	33.0%	72.0%
III	46 mice	39 tumor mice = 88.0%	52.0%	

The Total Hybrids Cream + (European + I daughter of No. 10)
(three different crosses used)

I	498 mice	22 tumor mice = 4.4%	8.4%	
II	365 mice	53 tumor mice = 15.0%	29.0%	27.5%
III	189 mice	62 tumor mice = 33.0%	62.6%	

In this case the tumor rate is again on the whole intermediate and slightly nearer that of the Cream, but the tumor age is that of the Cream, contrary to what we found in the former hybrids. If we omit from the table of hybrids one cross in which the tumor rate was low, the figures for the Cream + 10 hybrids with medium tumor rate (2 crosses) are as follows:

Total Cream + (European + I daughter of No. 10) hybrids with medium tumor rate

I	370 mice	20 tumor mice = 5.4%	10%	
II	286 mice	49 tumor mice = 17.0%	32%	34%
III	161 mice	56 tumor mice = 31.0%	58%	

Again the tumor rate is intermediate and the tumor age is almost that of the Cream. In this connection it is of interest to remember that the tumor age of the European 151 + I or II daughter of No. 10 is later than that of the English, and accordingly it has a tendency to transmit to crosses a later tumor age than the English.

If we cross strains in which tumor rate and tumor age differ less than in the preceding strains, we again obtain a tumor rate and tumor age intermediate on the whole, or perhaps resembling slightly more one parent than the other. This is shown in the following crosses:

A. German

I	42 mice	8 tumor mice = 19%	15.5%	
II	19 mice	7 tumor mice = 37%	30.3%	40.5%
III	3 mice	2 tumor mice = 66%	54.2%	

In this case the number of mice is rather small; we can therefore use it only with caution.

B. Total No. 8

I	243 mice	17 tumor mice = 7%	12.0%	
II	150 mice	24 tumor mice = 16%	27.4%	27.5%
III	78 mice	26 tumor mice = 35%	60.6%	

C. Total No. 8 + German

I	373 mice	30 tumor mice = 8%	14.0%	
II	280 mice	66 tumor mice = 24%	42.0%	34.0%
III	123 mice	31 tumor mice = 25%	44.0%	

The tumor rate of the hybrids is about intermediate and the tumor age is very similar in both parent strains as well as in the hybrids.

d. Another strain may be of interest; this resembles in its character the last named hybrid strain and is related to it. We crossed the 8 + German with an English male, the offspring of Tumor mouse 198.

The data for the parent strains have already been given. The figures for the cross are as follows:

English (198) + (8 + German)

I	217 mice	61 tumor mice = 28%	20%	
II	89 mice	52 tumor mice = 58%	41%	54%
III	9 mice	5 tumor mice = 55%	39%	

In this case the tumor rate of the hybrids is again intermediate, but slightly nearer that of the English. The tumor age is also intermediate, but again somewhat nearer that of the English. In this case no distinct split between tumor rate and tumor age occurred in the hybrids.

25. We believe that in these investigations we have established a definite relation between tumor incidence and tumor age. In general the average tumor age is the earliest in those strains of mice in which the number of individuals affected by cancer of the breast is greatest. There is in addition a difference in the tumor age in a number of strains in which the tumor rate is similar; or, conversely, in a number of strains in which the tumor age is similar the tumor rate may differ. We have seen furthermore that in crosses tumor age and tumor rate may be inherited independently of each other, or in other cases the tumor age may correspond to the tumor rate. How can these facts be interpreted in accordance with the current theories of heredity? We may assume as most probable that the tendency to cancer depends on the presence of multiple factors.

A certain number of these factors must be present in an individual if a tumor is to appear. These factors determine the intensity of the tendency towards cancer in each individual; but this intensity tends not only to cause a tumor to appear in a certain animal; but it has the tendency to make it appear at an early period of life. Tumors appear the earlier, therefore, the greater the number of tumor factors present or the greater the importance of the factors represented. We may assume that different strains differ greatly in the number and character of these factors present in the average of the individuals. In the English and $8\frac{1}{2} + 328$ there may be in an individual affected with cancer, on the average, a greater number of the more effective factors than in a Cream individual which happens to have just a sufficient minimum of factors to insure the appearance of a tumor. Therefore the intensity in the tendency towards the appearance of tumors is greater in the English and $8\frac{1}{2} + 328$ than in the Cream. In all these individuals tumors appear, but the greater intensity in the English and $8\frac{1}{2} + 328$ causes the tumors to appear earlier in life in these latter strains. There are, of course, variations in the individuals of different strains, and some individuals of the $8\frac{1}{2} + 328$ or English may resemble the average Cream as far as the character and number of their tumor factors are concerned. There may be, in addition, among the multiple factors determining tumor growth, some special factors which determine more directly the tumor age, and these special factors may be inherited independently of the multiple factors which determine mainly the appearance of a tumor.

These special factors may overcome the effect of the ordinary tumor factors, which in themselves already have a tendency to affect the tumor age. If, on the other hand, we assume that factors determining tumor age are always distinct from those determining tumor incidence, then we must conclude that both sets of factors are usually linked to each other in such a way that, in general, in those strains in which the tumor rate is high the factors determining an early appearance of tumors are also present; but that it is possible to dissociate these two sets of factors in certain cases, especially through hybridizations. Both interpretations are related to each other; the first interpretation including the second one. These interpretations must at present, of course, be considered as merely of atentative character, but they seem best to represent the facts so far established.

CONCLUSIONS

1. The tumor age of a certain strain is as definitely determined by heredity as the tumor incidence; the tumor age may be a finer means of distinction between different strains than the tumor incidence.

2. There is a definite relation between tumor rate and tumor age in mice. In those groups or strains in which the tumor incidence is great the tumors tend to appear early, and in those groups or strains in which the tumor rate is low the tumors tend to appear late. We can arrange our mice in three groups, with high, medium, and low tumor rate; in these three groups the tumor age shows corresponding changes.

3. The period of life at which a certain kind of tumor shows a maximum frequency in a certain species is not definitely fixed, but it varies with the rate of tumors in certain strains. The usual statistics represent an average between the maxima in different strains in which the maximum varies in accordance with their tumor rate.

4. In addition to this general relation between tumor age and tumor rate, there is in certain strains a specific tumor age which may differ from that expected in the strain on the basis of its tumor rate. Furthermore, in hybrid strains tumor rate and tumor age may be inherited independently of each other.

5. These relations between tumor age and tumor rate can best be explained if we assume that the hereditarily transmitted constitution, so far as it represents the tendency of the organism to develop tumors, depends on the coöperation of multiple factors. These multiple factors determine the intensity in the tendency to tumor development in a certain individual. In general, the greater is this intensity, the earlier do the tumors appear and the greater is the probability that in related individuals there exists likewise a tendency to the development of tumors.

It is furthermore probable that in addition to the general factors determining the intensity in the tendency towards the development of cancer, there exist factors which determine specifically the tumor age in certain individuals and strains.

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FURTHER INVESTIGATIONS OF DISTURBANCES OF BLOOD SUGAR EQUILIBRIUM IN THEIR RELATION TO NEOPLASIA[1]

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In previous publications (1), it has been shown that the injection of a protein into the animal body is followed by disturbance of the blood sugar equilibrium, and that this disturbance of sugar equilibrium can be influenced (either increased or decreased) by the ablation of one or more of the endocrines (2). The present report concerns a further study of: (*a*) the interrelationship of the endocrines in the regulation of blood sugar equilibrium; (*b*) the relation of the degree and type of blood sugar disturbance after the injection of protein to the strength of the anti-bodies developed; (*c*) the relation of the type and intensity of the reaction following the injection of homologous protein to neoplasia in man.

I. EXPERIMENTAL PART

The technical steps of the method employed are simple. The animal to be tested is starved for a period of twelve hours. Just before, and again sixty minutes after the subcutaneous injection of the antigen chosen, the blood sugar values are determined in milligrams per 100 cc. The lower of the two values is subtracted from the higher, the difference being taken to indicate the degree of disturbance as measured in milligrams. In the present work, in determining the blood sugar values we have used either the method of Epstein or of Kliner, or the Wallace and Gallagher modification of the Folin-Wu method. We have modified the Wallace and Gallagher method in that the blood is measured instead of weighed, 0.1 cc. being used. For this purpose a standardized pipet, so made that the capillary bore necessary to contain the specified amount is about 15 cm. long, was employed. It is essential that the same worker make all determinations in a given experiment and that the individual be skilled in the technical steps of the method chosen. The same method for determining the sugar values should be used throughout any one experiment. These conditions have been complied with in the work recorded here.

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[1] We beg to express our appreciation to all of the attending physicians and surgeons of the Lenox Hill Hospital for the clinical material contained in this report.

In our early experiments it was found that in normal animals after the injection of a given antigen, different types and degrees of reaction occurred. For example, one animal might show an increase of 100 mgm. in the blood sugar, while a second treated exactly as was the first might show an increase of but 15 mgm. A third animal, on the other hand, instead of showing an increase might show a decrease, for example, of 25 mgm. Hereafter, we shall use the terms "plus type" and "minus type" to designate, respectively, reactions in which the sugar concentration rises, and reactions in which a decrease occurs.

In investigating these variations the first factor considered was the influence of sex and age upon the type of reaction. A number of young female, young male, old female, and old male rats were tested in accordance with the method previously outlined, the injected antigen being 0.5 cc. of a 1 per cent heterologous protein (beef peptone). The averaged results of this experiment show that in the old males the plus and minus reactions occurred with the same degree of frequency, while in young and old females and in young males the plus reactions slightly predominated.

In a second experiment, one or another of the endocrine glands was removed, the test being performed at varying intervals after ablation. A 1 per cent heterologous protein (beef peptone), in doses of 0.5 cc., was first used as antigen, and after an interval of several days a similar dose of a similar strength of homologous protein (rat spleen) was employed. The glands removed were as follows: both testes complete, both ovaries complete, spleen complete, thymus complete, thyroid and embedded parathyroids about 95 per cent entire; both adrenals complete; pancreas about 90 per cent entire. As controls, normal animals and animals from which one kidney had been removed were used. There were from 6 to 12 animals in each group.

The data of the experiments are given in table 1. The percentages of plus reactions are about the same in the normal controls, in those with one kidney removed, and in the thymus-free, pancreas-free, and ovary-free groups, irrespective of which antigen was used. There was a difference in the types observed in the spleen-free group, for of these 16 per cent gave a plus reaction when injected with heterologous protein, as compared with 83 per cent giving a plus reaction when homologous protein was used as antigen. One type of reaction only was observed in three groups: the adrenal-free and thyroid-free groups gave 100 per cent plus reactions, and the testes-free 100 per cent minus reactions when injected with heterologous protein. When homologous protein was used as antigen the adrenal-free and testes-free groups reacted in the same manner, while the thyroid-free groups gave 100 per cent minus instead of plus reactions.

TABLE 1

ORGAN REMOVED	PERCENTAGE GIVING PLUS REACTIONS		PERCENTAGE GIVING MINUS REACTIONS	
	Homologous protein	Heterologous protein	Homologous protein	Heterologous protein
Normal controls	70	65	30	35
Kidney	60	55	40	45
Thyroid	0	100	100	0
Thymus	39	20	66	80
Spleen	83	16	16	83
Adrenals	100	100	0	0
Pancreas	58	67	42	33
Testes	0	0	100	100
Ovaries	78	75	22	25

Having observed the reactions after single gland removal, and in that manner having obtained groups in which all the animals reacted alike as regards type, we studied, in a third experiment, the effect of temporary overactivity of various glands. Extracts, in the proportion of 5 grams of the dried powdered gland in 25 cc. of physiological saline solution, were used as antigen, the dose being 0.5 cc. In this fashion extracts of the pituitary, pancreas, adrenal, and thyroid glands of sheep were prepared, and because these extracts, aside from their supposed specific secretion content, were also heterologous protein, a similar extract of dried sheep liver was used, as a control. The other details of the experiment were similar to those previously described. There were 12 non-operated rats in each group. The data of this experiment are given in table 2.

TABLE 2

GLAND EXTRACT INJECTED	PERCENTAGE PLUS REACTIONS	PERCENTAGE MINUS REACTIONS
Liver	58	42
Pituitary	50	50
Pancreas	25	75
Adrenal	42	58
Thyroid	67	33

In the animals injected with heterologous protein derived from the adrenal and thyroid glands there was little or no change in the percentages of the two reaction types as compared with the group injected with heterologous liver protein. When pancreas extract was injected, however, there was a predominance of the minus reactions, which is in contrast to the pancreas-free animals in which injections of heterologous protein produced a predominance of plus reactions.

The fact that, after single gland removal, but one type of reaction occurred in certain groups led to a study of the relationship of the endocrine chain in its effect upon the reaction types. Double gland ablations were done in such fashion that all possible two gland combinations were obtained, always excepting those with the pituitary.

Six rats were used in each group. At varying periods after operation these animals were injected with 0.5 cc. of homologous protein and sugar estimations were made as before. The data of the experiment are given in table 3.

When compared with single gland removal, double gland ablation showed the following changes in type of reaction. The spleen and testes combination showed results opposite to those of spleen and ovaries, plus types predominating with the former and minus types with the latter. The thymus-thyroid combination gave a predominance of plus types while the thymus-adrenal gave a majority of minus reactions. Three groups showed one type of reaction throughout; the adrenal-pancreas, testes-pancreas, and the thyroid-ovary combination gave 100 per cent minus reactions.

TABLE 3

	TESTES		OVARIES		SPLEEN		THYMUS		PANCREAS		ADRENAL	
	+	−	+	−	+	−	+	−	+	−	+	−
Thyroid	67	33	0	100	50	50	67	33	80	20	40	60
Adrenal	50	50	80	20	50	50	32	68	0	100		
Pancreas	0	100	25	75	60	40	52	48				
Thymus	60	40	67	33	60	40						
Spleen	75	25	25	75								

+ = plus reaction percentage
− = minus reaction percentage

As has been stated in a previous paragraph not only did normal animals differ in type of reaction, but they also differed in the intensity of the blood-sugar disturbances. If the intensity of the reaction, as measured by the difference in milligrams per 100 cc. of the two sugar estimations, be compared in the young and old (chart 1) it is found that the young react more distinctly than do the old. Of the two sexes the males react more distinctly than do the females.

If the intensity of the reaction which follows the injection of heterologous protein be compared with that which follows the injection of homologous protein in normal and in gland-free

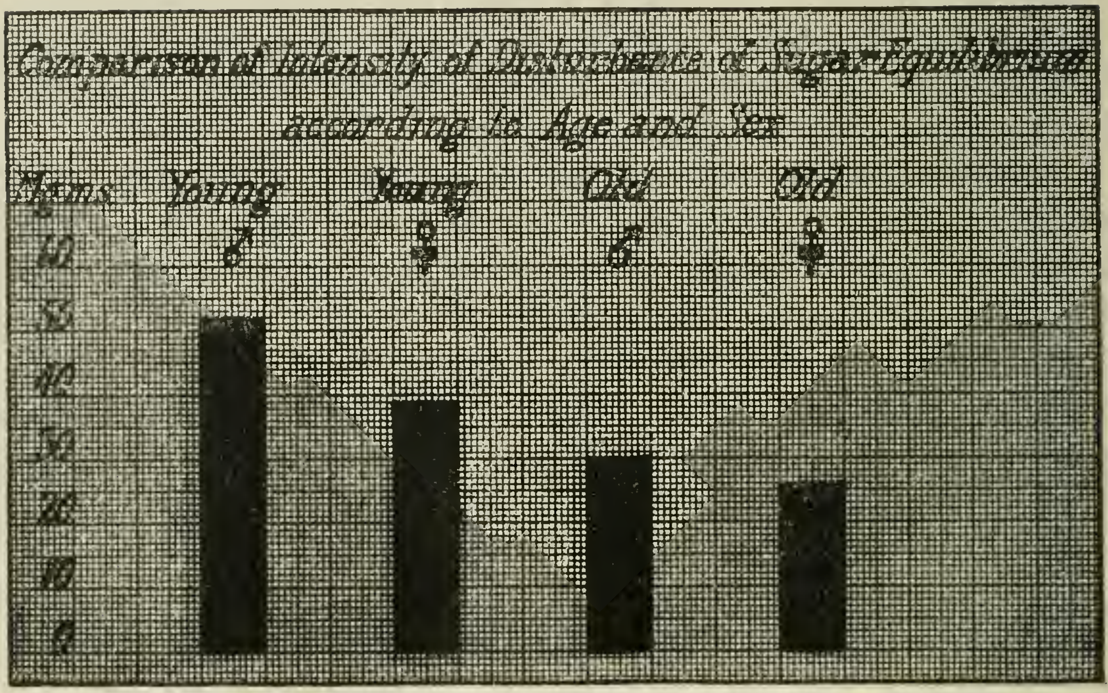


CHART 1

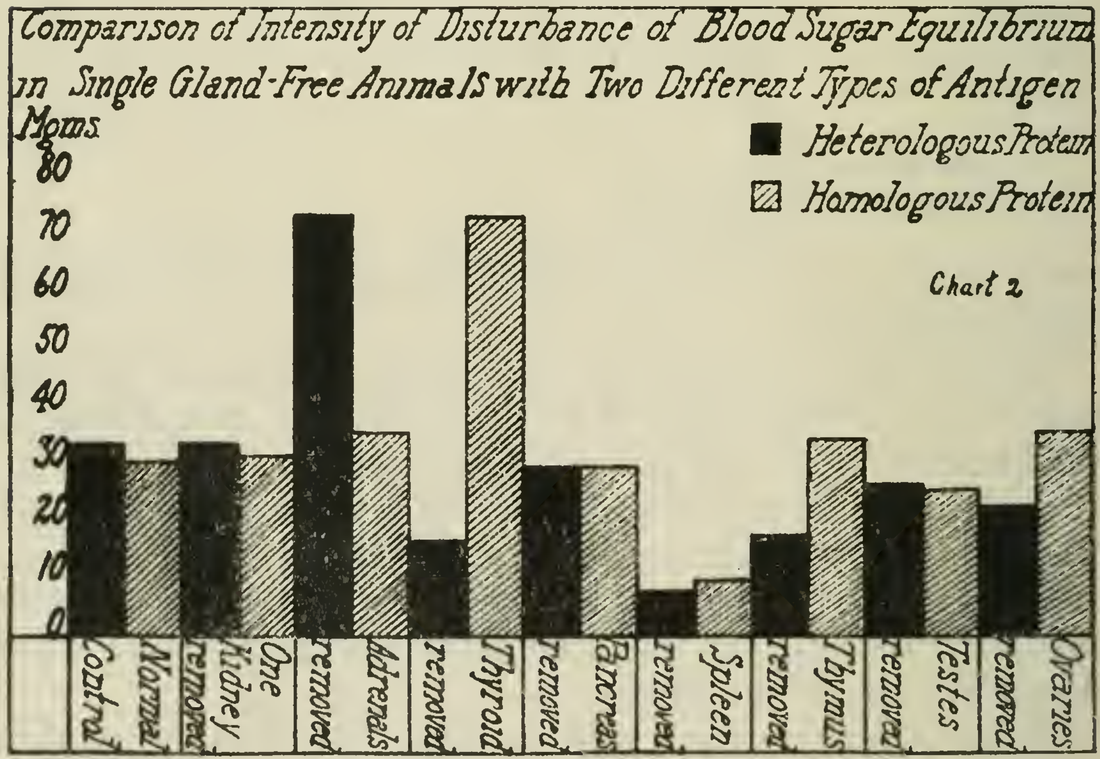


Chart 2

animals (chart 2), it is found that the same degree of reaction occurs with either antigen except in animals with the adrenals or the thyroid removed. The adrenal-free group reacted more distinctly to heterologous protein, while the thyroid-free reacted more intensely to homologous protein. Spleen-free animals showed a lessened intensity of reaction to both antigens.

When the reaction following injection of homologous protein is studied in animals from which two glands have been removed, the results (2), to quote from the paper previously referred to, are as follows: "There are two combinations of endocrines which when ablated inhibit the reaction; these are (1) spleen and either gonad set, and (2) adrenal and pancreas. If one gland from either system be ablated and with it one gland from the other system, there results little or no change from the normal reaction. If, however, one gland from one system be removed and with it one gland from another system, and in addition either the thyroid or thymus, then marked inhibition occurs. Removal of one gland from one system and either thyroid or thymus does not produce inhibition. If both glands be removed from each system, then, in order to induce inhibition, both of the intermediate glands, i.e., thyroid and thymus, must also be removed; the ablation of one intermediate gland is not sufficient. From this it appears that there are two systems which control the reaction and these systems are connected through two apparently interchangeable glands."

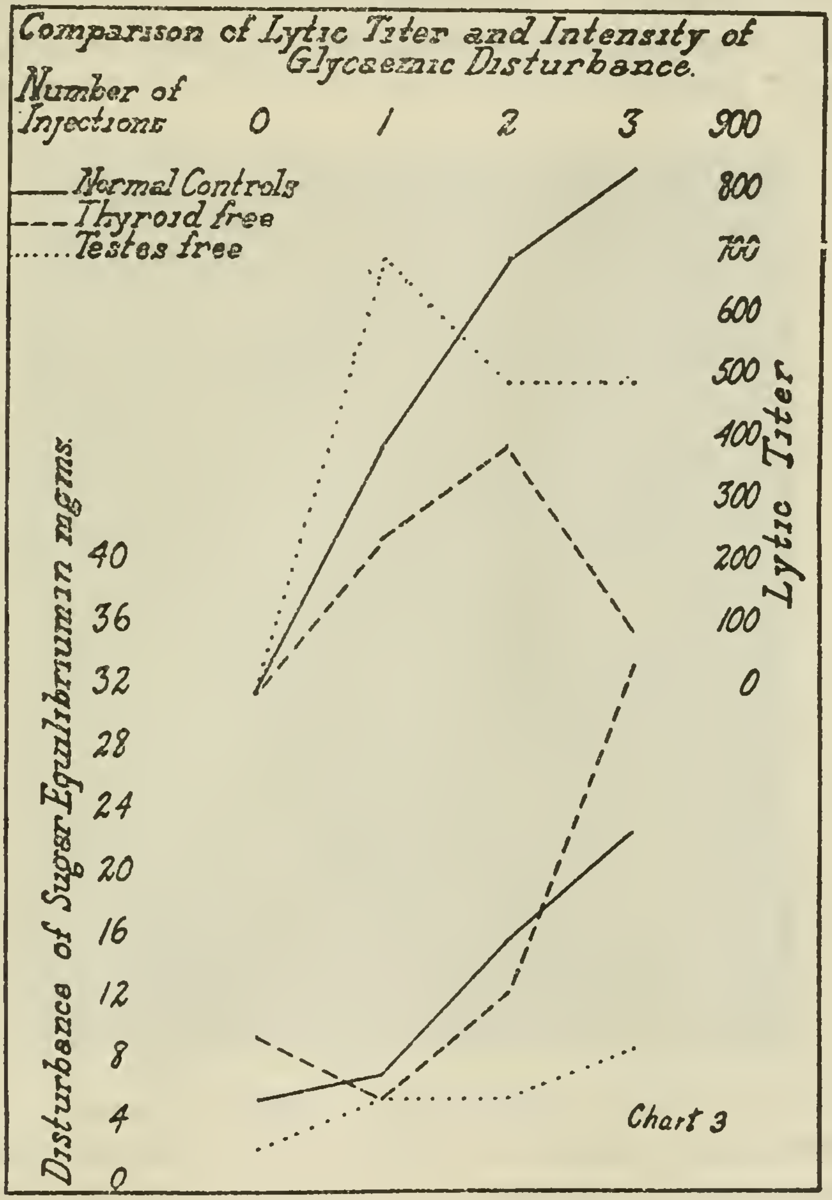
Stimulation of the reaction with double gland ablations occurred in two systems, analogous to those producing inhibition. These systems were the thyroid in combination with either ovaries, pancreas, or spleen, and the pancreas in combination with either testes, thyroid, or spleen.

The exact significance of variations in type and in intensity of reaction is somewhat in doubt. It might be supposed, since disturbances of blood-sugar equilibrium follow the injection of an antigen until the injected animal has exhausted the power to produce further antibodies against the antigen injected, that variations in the type or in the intensity might be indicative of the relative strength of the antibodies formed.

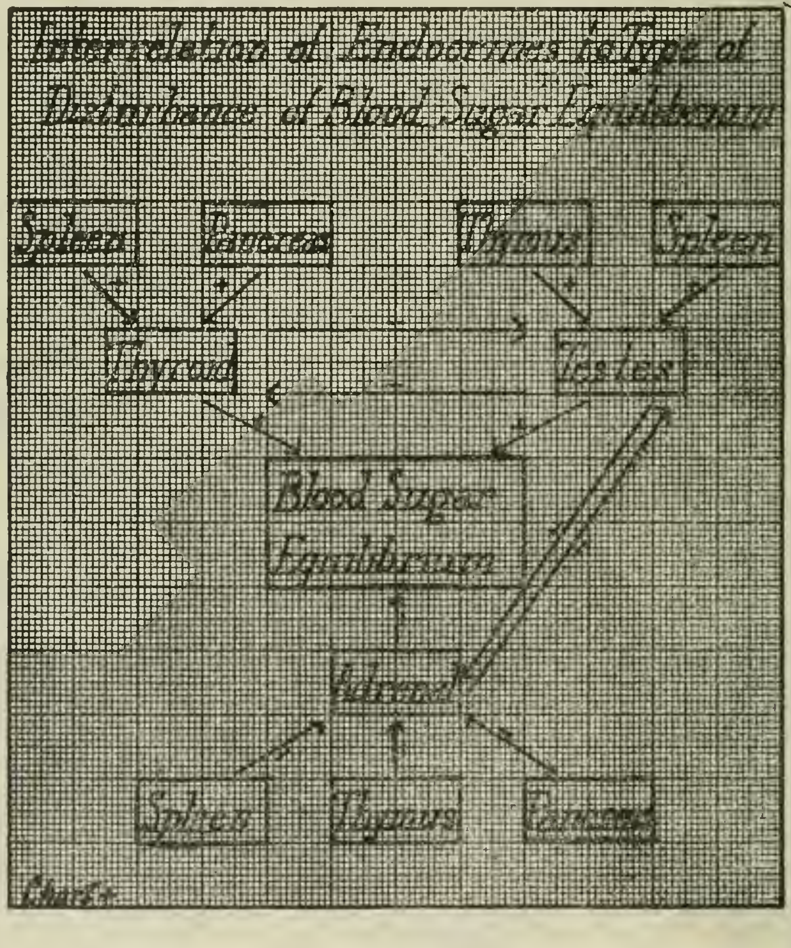
Whether such an hypothesis is valid or not was determined in the following experiment. Rabbits, in groups of four animals each, were prepared as follows: one group served as normal controls; from a second the testes were removed; while from a third the complete thyroid apparatus was excised. One week after operation and after twelve hours' starvation blood-sugar estimations were made, the animal was then injected intraperitoneally with 2 cc. of a 50 per cent suspension of washed sheep red cells, and one hour after the injection the blood sugar was again determined. Lytic titers were made with serum obtained from the first bleeding. Two subsequent injections and double sugar determinations as well as lysin titers were made at weekly intervals, and a final lytic titer was made one week after the last injection. As is shown in chart 3 the thyroid-free animals showed a greater sugar disturbance than did the control animals, and the control animals in time showed a greater disturbance than did the testes-free animals. If intensity of reaction bears relation to the strength of antibody formation then we should expect the thyroid-free group to show the highest lytic titer, and the testes-free the lowest titer. Such, however, was not the case, for the normal controls gave the highest titer, the testes-free next, and the thyroid-free the lowest titer.

There being no demonstrable relationship between the intensity of the reaction and the strength of the antibody development, the most plausible remaining explanation is that the phenomenon is an indication of the relative activity of various of the endocrines. The failure of the injected animal to react after the maximum antibody production has been attained might also be taken as an indication of the temporary exhaustion of the endocrines.

It is more logical, in endocrine studies, to consider averaged group behavior rather than individual behavior, and it is on averaged group behavior that the scheme of interrelationship given below has been worked out. If the ablation of a gland or of a group of glands is followed by a minus reaction, then that gland or the combination when in situ may be considered as causing a plus reaction. Similarly, if a given gland or gland group removal brings about an inhibition, then that particular combination when in situ could be considered as stimulators and vice versa. The deviation one way or another from the behavior of the control group of normals would serve in lesser degree to indicate further interglandular relationships.



Thus as is diagrammatically shown in chart 4, if the injection of a homologous protein is followed by a drop in the blood-sugar concentration, either the thyroid or, if the individual be a male, the testes, may be considered as inefficient; whereas, if the sugar values rise, the adrenals may be considered as overactive. It may also be supposed that decreases in the sugar concentration are due to lessened activity of the adrenal whose function is to keep up the sugar concentration. This insufficiency may be due either primarily to the adrenal or secondarily to the ineffectiveness of the stimuli reaching that gland through the spleen, thymus, or pancreas. In a similar manner, a rise in the blood-sugar concentration may be attributed to failure of the thyroid or testes to hold the blood sugar at the lower level, and this may in turn be due to the thyroid or testes primarily, or it may occur secondarily because of failure of the stimuli from either spleen, pancreas, or thymus.

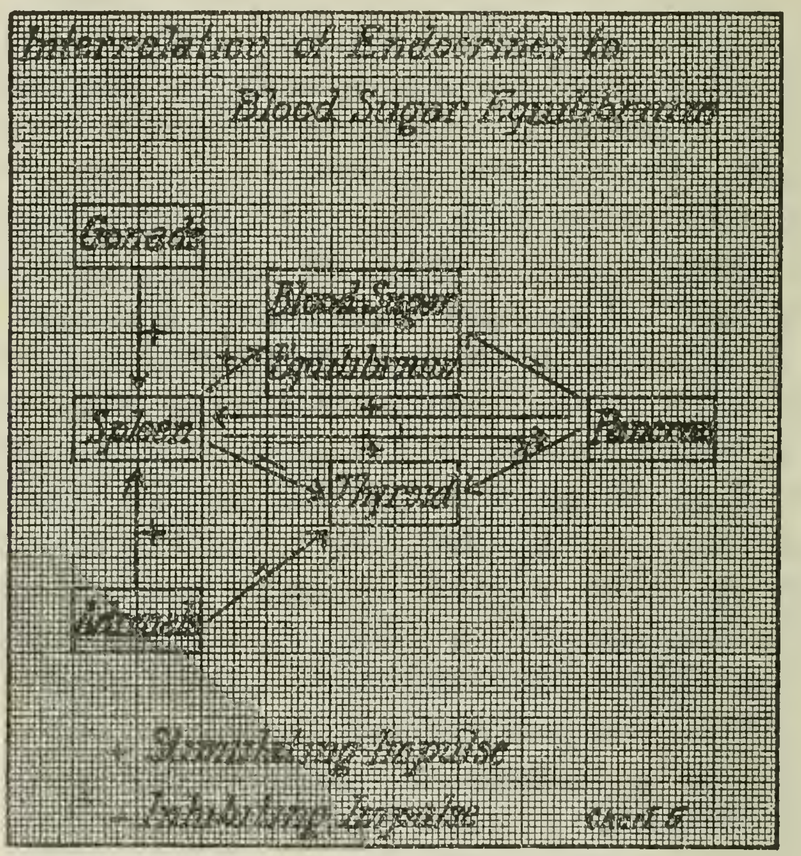


The intensity of the reaction has been worked out on similar lines. Accepting, for example, that a normal intensity of reaction for the rat is 31 mgm., if a gland removal results in reaction intensity of but 10 mgm. then the gland removed may be considered when in situ to be one of those helping to keep up the normal intensity. If two glands be removed from two different groups and it is found that group A has a reaction intensity of 45 mgm. and group B a reaction intensity of 10 mgm. it may be accepted that the gland whose removal brought about the increased intensity, is when in situ one of the group which keeps the intensity down, the opposite being the case in the group in which gland removal caused a less intense reaction. Now, if both glands be removed from a third group and the reaction intensity of this group is found to be 10 mgm., it may be supposed, gland A in situ being an inhibitor, and gland B in situ being a stimulator, that gland A sends an inhibiting influence to gland B, for gland A being removed permits gland B to act without restraint, and the reaction is intensified. Gland B being removed permits gland A or some other gland to act without restraint in consequence of which the reaction intensity falls.

The intensity of reaction according to glandular interrelationship is indicated in chart 5. For example, if the adrenal be removed, the spleen lacks a stimulating influence and the thyroid an inhibiting one; the retention of a normal reaction intensity might, therefore, be expected, for the plus and minus influences balance each other, the actual experimental values being: normal group, reaction intensity 31 mgm.; adrenal-free group, reaction intensity 36 mgm. Ablation of the spleen results in the removal of secondary stimulation received via the gonads, adrenal, and pancreas, and the removal of a stimulating impulse sent to the pancreas, as well as directly upon the blood-sugar concentration. It might be expected, therefore, that the splenic removal would depress the intensity of the reaction. The actual experimental values obtained were: normal animals, reaction intensity 31 mgm.; spleen-free group, reaction intensity 10 mgm.

Theoretically, therefore, if the blood sugar concentration after an injection of homologous protein falls distinctly, it may be supposed that the mechanism which causes increased concentration (chart 4) has not responded or that the mechanism which depresses has been stimulated. An intense reaction would speak for thyroid inhibition. In contrast, if the reaction were slight, say 10 mgm., splenic inhibition would be suspected. Inversely, if the sugar concentration rises distinctly it would indicate adrenal hyperactivity in combination with thyroid and pancreas, while if the reaction were minimal it would indicate adrenal hyperactivity in combination with spleen and gonad.

That disturbances of blood-sugar equilibrium no longer occur after the injection of protein, when the organism has attained its maximum antibody producing power is, on this basis, to be interpreted as indicating temporary exhaustion of certain of the endocrines.



CLINICAL APPLICATION

It has already been noted that a majority of the mice bearing spontaneous tumors subjected to this test have shown a decreased intensity of reaction. In order to follow this lead, further investigations were carried out on human beings suffering from a large variety of pathological conditions. The technic of the test as applied to the human cases was as follows:

After the evening meal no medication was given, the patient was starved until the last specimen of blood was withdrawn the following morning. Just before and again 45 and 120 minutes after the subcutaneous injection of 10 c.c. of homologous protein, blood was withdrawn and the sugar content determined. The degree of disturbance of equilibrium was estimated by subtracting the lowest from the highest of the three values obtained, the difference in milligrams being the standard of comparison. The homologous protein used was either blood serum or ascitic fluid obtained from individuals free from syphilis, tuberculosis, or cancer, in order to obviate any possible chance of disease transmission. The sterility of the fluid was assured by culture. No reactions were encountered as a result of such injections except in one instance where there was a slight chill followed by a rise in temperature to 102°F. With the Folin-Wu method we have found that not more than three unknowns can be compared with the same standard because the colors of the standard fade rapidly.

The clinical diagnoses have in every instance been confirmed either by histological or bacteriological examination, autopsy, operation, or investigation of the subsequent course of the patient. All the cases of neoplasia have been confirmed microscopically.

Before proceeding to an analysis of the data obtained as a result of these investigations it is profitable to revert to those obtained during investigations already reported (3). In this previous investigation blood-sugar equilibrium was disturbed by the oral administration of 100 grams of anhydrous glucose. When the individuals examined by this method were divided into male and female, cancerous and noncancerous, the following results were obtained. There was no predominance of type in one group over another, i.e., a particular type of reaction could not be said to be characteristic of any given group. If the intensity of the reaction, i.e., the degree to which blood-sugar equilibrium was disturbed was compared (chart 6) it was found that with the exception of the non-cancerous males all the groups gave the greatest percentage of cases with a reaction intensity of from 61 to 120 mgm.; in brief, there was nothing characteristic of neoplasia.[2]

However, when the data of the types and intensities of reaction observed after the injection of homologous protein in man were similarly analyzed there were some very interesting and suggestive variations.

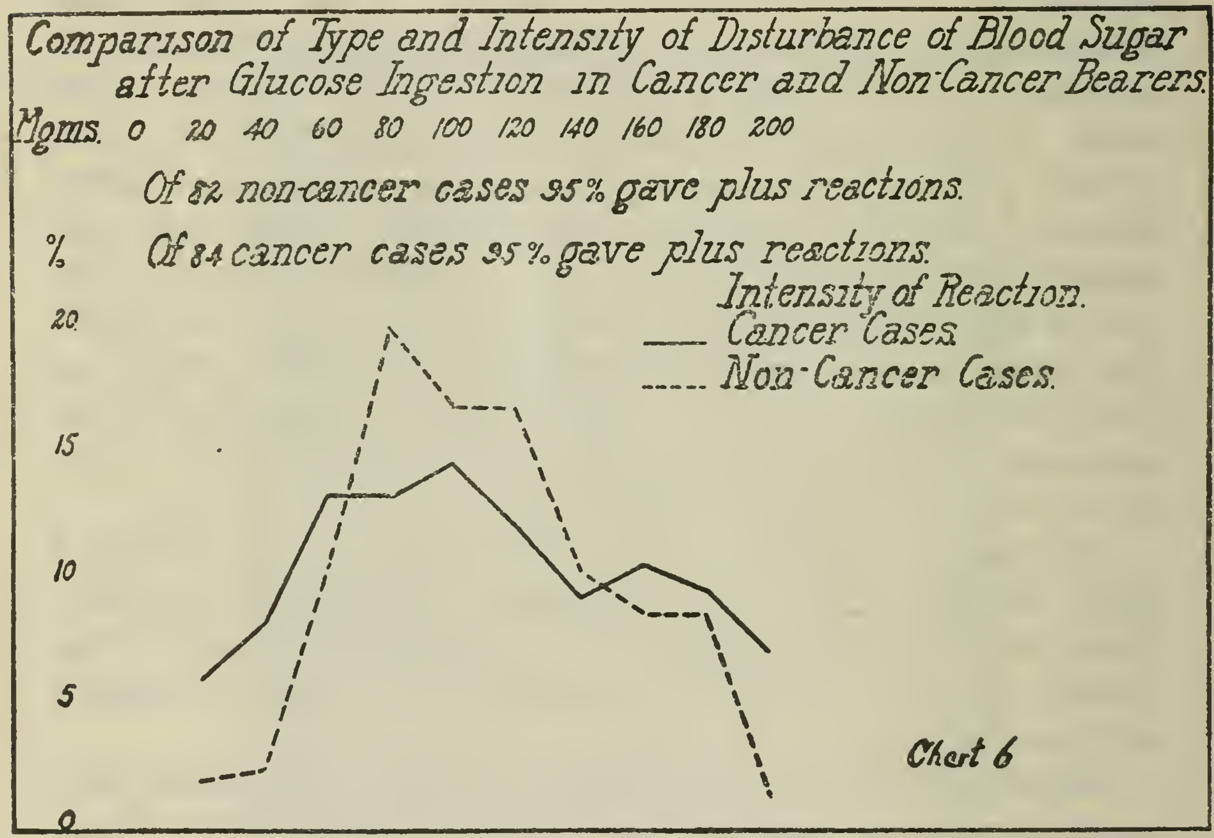


Table 4 gives the statistical data of the 209 cases which have been examined. When this information is presented as in table 5, several points are evident; a larger percentage of the

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[2] In a recent publication Killian and Kast (4) stated that the hyperglycemia observed in cases of neoplasia is attributable to the nitrogen retention present in many of these cases. This factor had not been considered by us in our previous communications. A review of our data, however, does not bear out the contention advanced by these authors, but confirms in a very emphatic manner the observation made by Spence (5) who attributed the hyperglycemia to the age of the individuals. In his investigations Spence showed that hyperglycemia occurs with increasing frequency in the more advanced age periods.

TABLE 4

CASE NUMBER	DIAGNOSIS	DISTURBANCE OF BLOOD SUGAR
		mgm.
6432	General carcinomatosis	−9
S2740	Carcinoma rectum	−9
177	Carcinoma lung	+4
6977	Carcinoma stomach	−3
7088	Multiple myelomata	+4
7118	Carcinoma stomach	−12
92	Carcinoma cecum	−6
54	Carcinoma pancreas	−10
262	Carcinoma rectum	+6
391	Carcinoma gall bladder	+5
528	Carcinoma breast	+3
794	Melanocarcinoma skin	+4
1260	Carcinoma stomach	−2
1542	Sarcoma lung	+7
1573	Carcinoma stomach	+5
1659	General carcinoma	+4
1735	General carcinoma	+5
1563	General carcinoma	+6
1820	Sarcoma femur	+10
1761	Carcinoma colon	−4
1853	Carcinoma stomach	−5
1944	Carcinoma rectum	+7
2165	Carcinoma general	+9
2192	Carcinoma stomach	−11
2465	Carcinoma stomach	+10
3056	Carcinoma general	+4
2892	Carcinoma general	+10
3152	Carcinoma stomach	−3
2942	Carcinoma rectum	+4
3752	Carcinoma stomach	−5
3892	Carcinoma cervix	+5
4647	Carcinoma stomach	+7
5442	Carcinoma general	+4
P2386	Carcinoma stomach	−9
P2306	Carcinoma stomach	+12
P2488	Carcinoma sigmoid	+7
6368	Carcinoma bladder	+6
6479	Carcinoma stomach	+5
P2820	Sarcoma ovary	−5
6950	Carcinoma vagina	+3
831	Carcinoma bladder	−6
1235	Carcinoma prostate	+3

TABLE 4—*Continued*

CASE NUMBER	DIAGNOSIS	DISTURBANCE OF BLOOD SUGAR
		mgm.
1353	Carcinoma stomach	−5
1273	Carcinoma liver	+3
1587	Carcinoma stomach	−10
1825	Carcinoma stomach	−10
1869	Carcinoma esophagus	−6
2114	Carcinoma rectum	−6
2305	Carcinoma rectum	+10
2625	Carcinoma breast	−8
2709	Carcinoma generalized	−10
2471	Carcinoma generalized	−2
3604	Carcinoma cervix	+4
3517	Carcinoma breast	−6
3627	Carcinoma liver	+9
3021	Carcinoma esophagus	+6
3858	Carcinoma colon	+6
1740	Carcinoma esophagus	−6
3421	Sarcoma parotid	+4
3533	Sarcoma pelvis	+2
3836	Sarcoma retroperitoneal	+4
4197	Carcinoma stomach	−8
4213	Carcinoma breast	−5
4216	Carcinoma rectum	+8
225	Carcinoma stomach	+2
4510	Carcinoma stomach	−6
4134	Carcinoma stomach	+4
4414	Epithelioma cheek	−4
3097	Carcinoma stomach	+10
14149	Carcinoma stomach	+6
4747	Carcinoma stomach	+3
4565	Carcinoma stomach	−6
4493	Carcinoma stomach	−2
4708	Carcinoma larynx	−4
5113	Carcinoma stomach	+3
4748	Carcinoma cervix	+4
2590	Carcinoma intestine	+12
128	Carcinoma liver	−6
5193	Carcinoma cervix	−12
4531	Carcinoma tongue	−10
5821	Carcinoma breast	+12
6044	Carcinoma colon	+9
3225	Carcinoma breast	+10
5508	Carcinoma sigmoid	+8

TABLE 4—*Continued*

CASE NUMBER	DIAGNOSIS	DISTURBANCE OF BLOOD SUGAR
		mgm.
5836	Carcinoma stomach	−3
5839	Carcinoma breast	−9
158	Carcinoma stomach	+6
6153	Carcinoma esophagus	+6
754	Retroperitoneal sarcoma	−33
700	Carcinoma esophagus	−35
2300	Carcinoma stomach	−51
2315	Carcinoma lung	−51
3605	Carcinoma esophagus	+54
69	Sarcoma tendon sheath	+20
80	Carcinoma colon	+36
4936	Carcinoma stomach	−34
123	Epithelioma penis	−42
5637	Carcinoma uterus	−27
5793	Carcinoma uterus	+30
3728	Lymphosarcoma neck	+28
5746	General carcinoma	−34
6116	Carcinoma ovary	−33
6049	Carcinoma bladder	−31
6347	Carcinoma mouth	+30
1807	Carcinoma general	−21
1757	Carcinoma appendix	+19
2768	Carcinoma uterus	+22
3202	Carcinoma testes	−20
3560	Carcinoma stomach	+49
266	Carcinoma lip	+14
4965	Endothelioma brain	+30
594	Syphilis	−48
797	Inguinal hernia	−44
864	Encephalitis	−24
835	Biliary cirrhosis	−15
1102	Encephalitis	−16
1219	Syphilis	+21
1379	Neuritis	+42
770	Tuberculosis	+15
1580	Achylia gastrica	+15
1373	Gastric ulcer	−21
1299	Orchitis	+15
2222	Varicose veins	+18
2552	Leukemia	−42
2641	Fibroadenoma breast	+18
30	Cryptorchidism	−39

TABLE 4—*Continued*

CASE NUMBER	DIAGNOSIS	DISTURBANCE OF BLOOD SUGAR
		mgm.
31	Cryptorchidism	−20
32	Angioneurotic edema	−18
36	Hyperthyroidism	+28
37	Retinitis pigmentosa	+45
3209	Pernicious anemia	+28
3889	Pernicious anemia	+36
3822	Orchitis	−32
4464	Ovarian cyst	+29
2467	Endocarditis	+21
4792	Myocarditis	+28
4825	Cardio-nephritis	+48
4937	Lymphadenitis	+62
4943	Fibromyoma uteri	−40
5121	Endocarditis	+35
126	Gastritis	−36
5244	Postoperative adhesions	−18
5197	Pregnancy	−18
5356	Hypertrophied prostate	+34
5312	Fistula in ano	+16
5407	Catarrhal jaundice	+39
5452	Graves' disease	−33
5319	Pernicious anemia	−22
5645	Subacute gastritis	−24
5582	Catarrhal jaundice	−17
5628	Endometritis	−39
5654	Oophoritis	+24
5830	Neurasthenia	−17
5842	Gastric ulcer	+20
5814	Splenomegaly	−63
6115	Cholelithiasis	−22
6046	Mixed tumor parotid	+23
5406	Cerebral thrombosis	−53
6530	Diabetes	+60
6603	Uterine fibroids	+45
6841	Papilloma bladder	+22
237	Cholelithiasis	−42
3183	Diverticulitis	−15
527	Gastric ulcer	−28
583	Neurasthenia	+25
100	Tuberculosis	+16
1465	Ovarian cyst	+15
1109	Cholecystitis	+33

TABLE 4—*Concluded*

CASE NUMBER	DIAGNOSIS	DISTURBANCE OF BLOOD SUGAR
		mgm.
1639	Pyelitis	+22
1604	Cholecystitis	+18
1809	Sciatica	−15
1827	Chronic inflammation	−14
478	Gastric ulcer	+16
2554	Tuberculosis	+20
2588	Chronic cholecystitis	+18
2689	Gastric neurosis	+15
3229	Cholecystitis	−17
5436	Angina pectoris	−39
6843	Gastric ulcer	−38
5564	Lung abscess	−15
1511	Pernicious anemia	−9
35	Pregnancy	+6
5058	Gastric ulcer	+4
5372	Cystocele	+10
5401	Duodenal ulcer	+6
141	Orchitis	+10
5565	Syphilis	+10
5727	Cholecystitis	+
6016	Duodenal ulcer	+10
6083	Pernicious anemia	+9
6943	Chronic appendicitis	−7
7123	Nephritis	+11
136	Bronchopneumonia	−8
198	Fibroid uterus	+10
7086	Fissure in ano	+2
984	Prolapse uterus	+1
1008	Diverticulitis	+10
369	Duodenal ulcer	+2
1306	Fibroid uterus	+2
1873	Nephritis	−9
2293	Cholecystitis	+5
3121	Chronic endocervicitis	+4
3351	Cholecystitis	+3
3665	Tuberculosis	+4
3205	Hepatitis	−7
4440	Cholelithiasis	−5
4421	Polyp of sigmoid	+3
5570	Hypertrophy of prostate	+7
201	Septic endocarditis	+2
3566	Pernicious anemia	+9

cases of neoplasia of the gastro-intestinal tract and of the liver and gall-bladder show reactions of an intensity of less than 12 mgm., than is the case in individuals who have non-malignant diseases of these organs. In diseases of the genito-urinary apparatus this relationship does not apparently hold true, but in the series of miscellaneous neoplasias, no one type being present in sufficient numbers to warrant separate tabulation it is again evident that a larger proportion of the cancerous individuals show a lessened intensity of reaction than do a similar group of non-cancerous individuals.

TABLE 5

	REACTION	
	Less than 12 mgm.	More than 12 mgm.
Neoplasia of genitourinary tract	7 (54%)	6 (46%)
Neoplasia of gastrointestinal tract	50 (88%)	7 (12%)
Non-grouped neoplasia	29 (74%)	10 (26%)
Non-malignant disease of genitourinary tract	9 (41%)	13 (59%)
Non-malignant disease of gastrointestinal tract	13 (38%)	21 (62%)
Non-grouped non-neoplastic diseases	9 (20%)	35 (80%)
All cases of neoplasia	86 (78%)	23 (22%)
All cases non-neoplastic	31 (31%)	69 (69%)

These differences are distinct when compared with the similarity of behavior found in our previous series where blood-sugar equilibrium was disturbed by the ingestion of glucose. The observation is possibly of value as a diagnostic procedure. In diseases involving the gastro-intestinal tract, from mouth to anus, duodenal ulcer excepted, and in diseases involving the gall-bladder and liver, 88 per cent of all the cases of neoplasia, i.e., 50 of 57 cases, showed a reaction of less than 12 mgm. As regards the reaction types it was found that 39 per cent of the neoplasia cases gave plus reactions as compared with 52 per cent in the non-cancerous group. If the previously outlined endocrine relationship be accepted, such lessened reaction would indicate either spleen, adrenal, or gonad insufficiency, or thyroid, adrenal, and pancreas overactivity.

CONCLUSIONS

1. The disturbance of blood sugar equilibrium which follows the injection of a protein varies in intensity and in type.

2. The removal of certain of the endocrines influences both the intensity of the reaction and its type.

3. Neither the intensity of the reaction nor the type have any relation to the strength of the antibody development after the injection of a selected protein.

4. A study of the type and reaction intensity has been used in an attempt to establish paths of endocrine correlation.

5. Human cases of neoplasia show a larger proportion of weak sugar reactions, i.e., of 12 mgm. or less, than do non-cancerous individuals. This is particularly true of neoplasms of the gastro-intestinal tract and liver.

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PREGNANCY AND TUMOR GROWTH

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The relationship between pregnancy and tumor development has been investigated by a number of observers, whose results have been, however, in many instances contradictory. For example, Moreau (1) and Herzog (2) found that transplanted tumors grow with unusual rapidity in pregnant animals. Haaland (3), on the other hand, maintained that pregnancy exerts a markedly restraining influence on the growth of transplanted tumors, which continue to develop in their usual fashion after parturition. Bashford and Murray (4) hold that "pregnancy" in the female "and full sexual activity in the male constitute no bar to successful transplantation." Uhlenhuth and Weidanz (5) observed retardation in the growth of tumors during pregnancy and also more frequent spontaneous retrogression. Bridré (6) noticed a low percentage of positive inoculations in pregnant animals, and Ehrlich (7) observed that inoculations into animals bearing young was frequently followed by negative results and that in those successfully inoculated the growth of the tumor was greatly retarded. Then, again, Albrecht and Hecht (8) maintained that pregnancy affects the growth of tumors just as little as the presence of a tumor influences conception or gravidity. Cuenot and Mercier (9) reported a most interesting observation. They noticed that Borrel's tumor "B," which rarely underwent spontaneous absorption, if inoculated before fecundation developed during gestation and receded during lactation. The tumor, however, did not regress if one mouse only was born, thus leaving the activity of the mammary gland at a minimum. Neither did absorption occur even in the presence of several young, if the tumor was so situated that its vascularization was independent of that of the mammary gland. Fichera (10) explained these various inconsistencies by assuming that when many embryos were present the food stuff was almost wholly consumed by them, while if there were only a few some of this material was available for the tumor cells.

Slye has concluded from her observations of spontaneous tumor growth in the mouse that: First, reproducing females grow much less tumor than do non-reproducing females of the same age, etc. Second, reproducing females grow much less tumor while they are reproductive than they do while they are non-reproductive. In other words, she finds that pregnancy exerts a markedly retarding influence upon growth of tumor.

In re-investigating this problem rats were employed. The animals selected were young adults, sexually mature, vigorous, and in very good condition, which had been separated from male animals for a period of one month to avoid the possibility of unrecognized pregnancies. The rats were mated with strong, vigorous young males that were kept in the same boxes not only during the entire period of gestation but also after the females had littered, in order to impregnate the same animals immediately after parturition. In all cases the date of conception was calculated by counting back twenty-one days before the date of littering. All the animals were inoculated on the same day with 0.003 gram of Flexner's rat carcinoma, the time chosen being two days after the animals had been mated. In all, 105 animals were inoculated. Of these, 43 (41 per cent) became pregnant and littered normally.

In analyzing the cases in which no growth occurred, it is interesting to note that about 7.8 per cent of the males inoculated showed no growth, and that about 8.4 per cent of the females showed no growth. In the group of females without tumor growth, 44 per cent did not become gravid. The other 46 per cent did. These figures tend to show that in the negative cases neither sex nor gravidity played any rôle.

Tracings of the tumors were made every four days, and in this way growth was depicted simply but effectively.

A comparison of the charts (figs. 1 to 4) of the tumors of the pregnant and of the non-pregnant groups shows at a glance that in these experiments pregnancy had absolutely no effect at all upon the growth of the transplantable Flexner rat carcinoma.

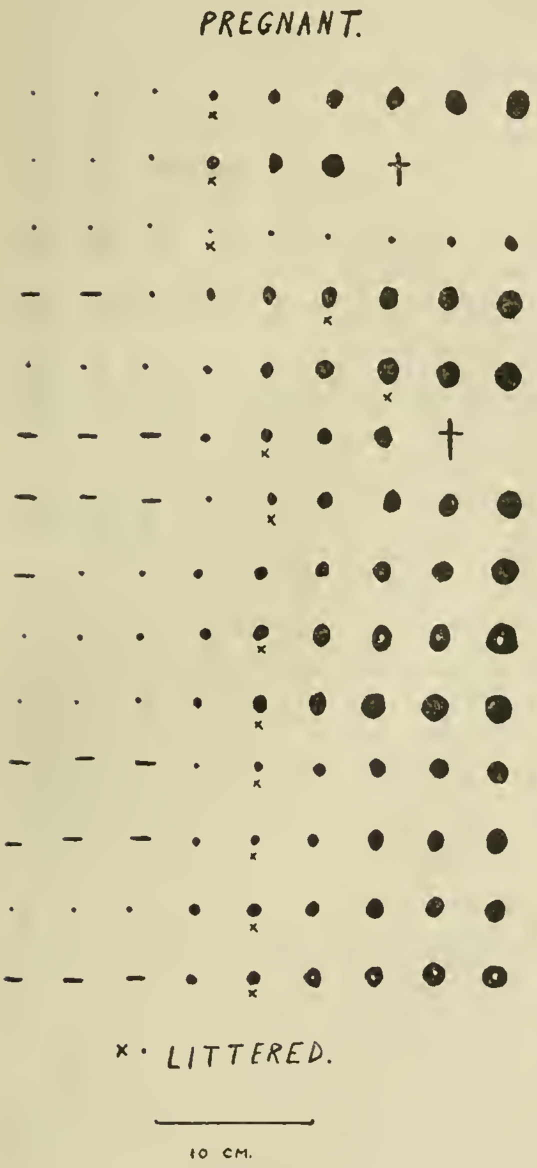

FIG. 1

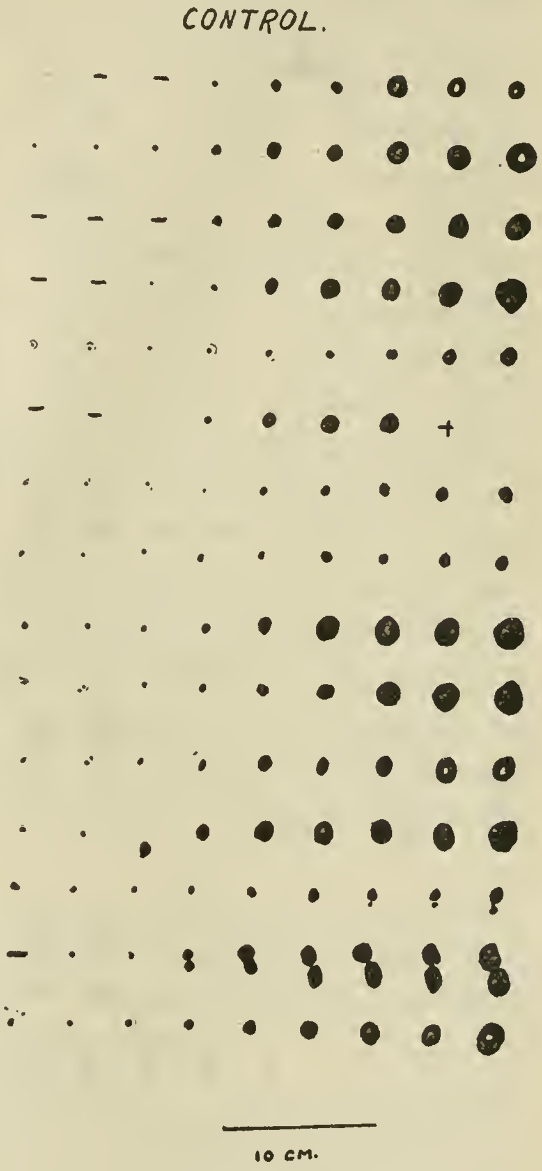

FIG. 2

DISCUSSION

It seems impossible completely to reconcile all of the conflicting results which have been published in regard to the effect of pregnancy on the growth rate of inoculated tumors, chiefly because we have insufficient data upon which to base any conclusions as to the growth capacity of the tumors which the various experimenters have employed. Nor do we know, in many instances, the site of inoculation. Mice are especially unsuitable animals for such a study, as the implanted tumors are apt to be

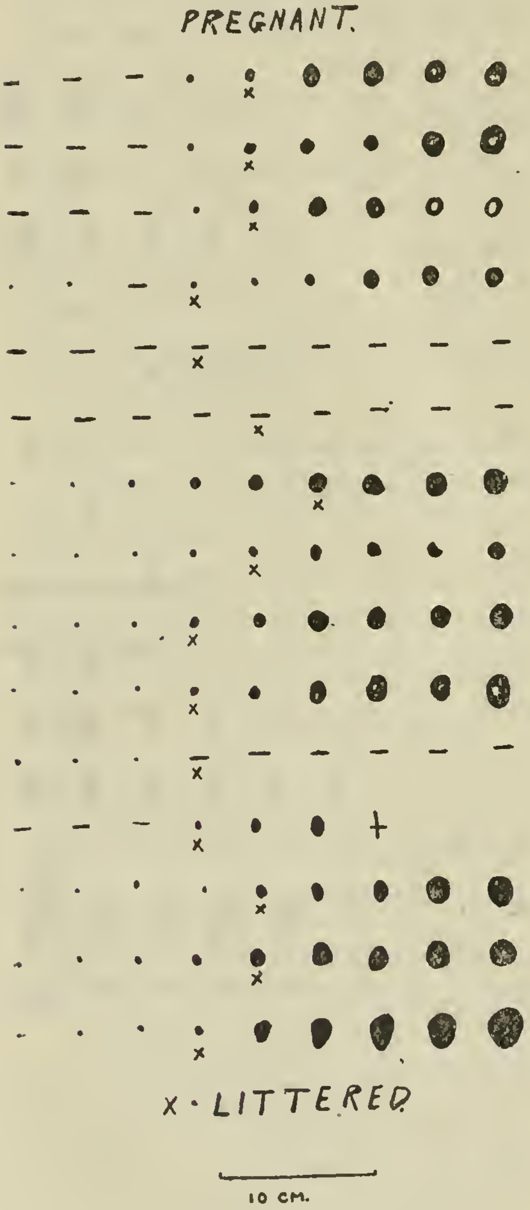

FIG. 3

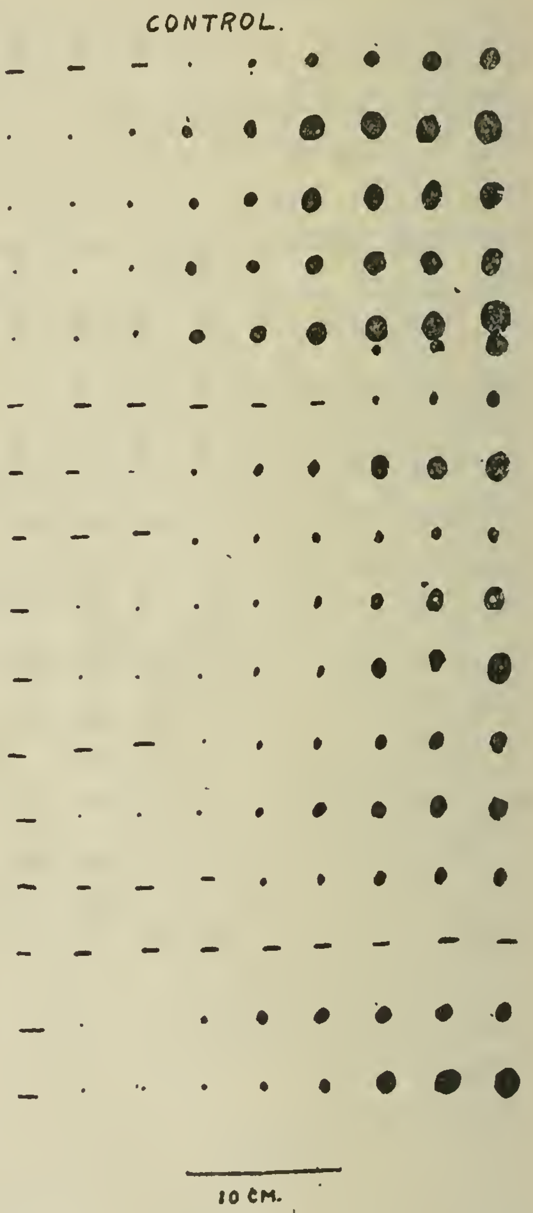

FIG. 4

variable in their growth rate, and may become so large as to kill the animal quickly. This obviously complicates the problem and renders careful control and statistical analysis of the results necessary. But it does seem possible to draw a few conclusions, not entirely inharmonious with the reported experimental work and the facts obtained by clinical observation.

The tumor grafts in the experiments here reported were not placed in the mammary gland but by intent in the axilla; they were, therefore, not affected by the increase in the vascularity of the breast at the beginning of pregnancy as they would be if placed directly in the main mass of the mamma. This increase in vascularity is undoubtedly the explanation of the rapid growth of tumors in the breast observed in human beings. But the conditions in the human female and in female mice are not comparable. A mouse weighing 15 to 20 grams will frequently carry a tumor weighing one-tenth of its weight. Unfortunately, the weights of the tumor-bearing animals are not given in Miss Slye's paper, though the dimensions of the tumors are furnished.

On the other hand, the weight of the tumor in a human female is rarely more than one one-hundredth or one one-hundred and fiftieth of the body weight. Mammary cancer in a mouse, therefore, may correspond in mass to a rapidly growing breast carcinoma of 10 to 15 pounds in a human female, a tumor which is practically never seen. It is quite possible that the demand for food for such a theoretical tumor if complicated by pregnancy might result in the starvation of the tumor for a time as Moreschi (12) has shown to be possible.

It is, therefore, evident that Miss Slye's results, while applicable to one species of mammalia under the conditions which she specifies, are by no means obviously transferable even to other types of rodents, much less to human beings.

The fact that in the additional experiments here reported no influence on the growth rate of tumors was shown is probably due to the disproportion between the size of the rat and its embryos, frequently weighing 150 grams with 25 grams of embryos, and the tumors, which are usually not more than one-fiftieth to one-hundredth of the weight of the animal. So, too, in mice the proportionate weight of the embryos demanding food is larger than that ordinarily occurring in the human female, a child rarely being over one-fifteenth of the mother's weight, while in the multiple mouse pregnancies 5 grams of embryos may be produced at a single birth from a mother weighing only 22 grams.

A grafted tumor, such as we have employed, being a mass of somatic cells growing in a foreign host, affords a possibility for studying the problem of tissue growth independently of organ or other relationships. The tumor is simply implanted in the subcutaneous connective tissue, while a primary tumor of the breast, in its inception at least, has important anatomical correlations with the tissue in which it grows. It is thinkable that some of the results observed by Miss Slye may be due to changes in the connective tissue stroma of the tumor rather than to the epithelium, but in the implanted tumor the connective tissue is furnished by the host, the epithelium being derived from another animal, so that the situation is quite different.

It seems safe to conclude, therefore, from our own experiments, that pregnancy of itself does not necessarily alter tumor growth rates, but that such interference, when it is observed, is due to the forced division of food substance between the tumor and the offspring; similar checking of the tumor can be observed in implanted tumors when the food of the non-pregnant animal is reduced approximately to the starvation point.

A comparable diminution in the tumor growth rate may be seen in human beings in the terminal stages of extreme cachexia with innutrition but, as a rule, in man pregnancy either has no effect on the progress of a cancer or, if the mammary or uterine tissues are involved, hastens the growth.

There is nothing, therefore, in the varying results of published experimental work which cannot be harmonized or which controverts the clinical observations already recorded.

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IS CANCER MORTALITY INCREASING?

WENDELL M. STRONG

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To determine whether or not there is a real increase in cancer mortality we must compare cancer death rates for the same ages, since cancer is an old age disease. The effect of age distribution is so great that it is safe to say that any considerable increase in the proportion of the population at the older ages, for instance, the emigration of the young men and women, would cause a noticeable increase in the cancer deaths per 100,000 population, while an influx of young men and women would decrease them.

Dr. Frederick L. Hoffman, statistician of the Prudential Life Insurance Company, in a paper on "The Menace of Cancer" published in 1913, and in his comprehensive book on "The Mortality from Cancer" published in 1915, has given the statistics available at the time of writing in a very complete form. These statistics were drawn both from the United States and abroad. From these I have selected a few.

In the registration of 1900, the cancer death rate per 100,000 population, which will hereafter be spoken of as the cancer death rate or cancer mortality, increased from 63 in 1900 to 79 in 1913, an increase of over 25 per cent. England and Wales for the same period showed an increase from 83 to 105 and most other European states showed a very considerable increase. Going back forty years, the rate in twenty large American cities was 49 for the period 1881–1885; for 1913 it had increased to 89. In twelve European countries the rate was 69 for the period 1896–1900; for the period 1906–1910, it had increased to 81. These were general population statistics without subdivision by ages. There were also available, however, some extensive statistics subdivided by ages. The cancer death rate in the registration states of the United States of 1900 subdivided into ten-year age groups, 25–34, 35–44, etc., for 1901 compared with 1911 shows an increase in the rate for each age group for the later year, such increase being very considerable for ages above 45, which are the significant ages in cancer. Massachusetts, for the two periods 1901–1905 and 1906–1910, with a different distribution into age groups and subdivided by sexes, showed an increase for each adult group and for each sex separately, in the later period the increase again being very considerable for the older groups. While these are but a few of the statistics, they are illustrative of the trend of practically all, and, standing alone, would appear to indicate a great and rapid increase in cancer mortality. We have, however, later and very extensive statistics from two of the great insurance companies which are not in accord with the above.

In 1919, Dr. Louis I. Dublin, statistician of the Metropolitan Life Insurance Company, published in this JOURNAL (1) the experience of that company on its millions of industrial policy holders for the years 1911–1916. Tables 1 and 2 are from this experience. They show fluctuations but no definite trend.

Table 1 is for the age group 55–64 only, which group Dr. Dublin chose as illustrative. We find in it the lowest mortality for white males in 1912 and 1915, the highest in 1913 and 1914; for white females the lowest in 1911 and 1914, the highest in 1912 and 1915. It is a curious accident that the years of low mortality for the one sex are in three cases the high for the other.

Table 2 compares the first two years of the period with the last two and in total shows but one per cent difference between them, a difference smaller than would be expected from merely accidental fluctuations. The two features which may be of significance are that the total of males shows an increase of 5 per cent and that the differences in each age group, as well as in the total of all ages (except perhaps 65–69), are no more than would be expected from accidental fluctuations.

The cancer experience for the Mutual Life Insurance Company for the years 1915–1920 has just been complied by Dr. Brandreth Symonds, chief medical director. This experience, given in table 3 below, is not subdivided by sexes, but as the great preponderance of insurance was on white male lives it may be taken as representing white male cancer mortality.

TABLE 1

Metropolitan Life Industrial Department. Cancer death rate per 100,000, ages 55 to 64

YEAR	ALL CLASSES	WHITE		COLORED	
		Males	Females	Males	Females
1916	386.4	358.0	427.4	218.3	339.9
1915	380.8	336.0	427.8	175.7	394.3
1914	390.9	385.0	423.3	167.7	351.7
1913	384.1	370.3	414.6	195.2	368.3
1912	381.9	334.1	443.2	176.4	325.4
1911	368.7	353.3	400.2	158.0	373.7

TABLE 2

Metropolitan Life Industrial Department. Ratio of cancer death rate for years 1915–1916 to death rate for years 1911–1912 by percentages

AGE PERIOD	ALL CLASSES	WHITE		COLORED	
		Males	Females	Males	Females
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
25 and over	101.0	105.2	98.3	105.1	100.7
25 to 34	98.0	121.0	94.1	95.5	95.0
35 to 44	100.1	104.3	96.9	142.6	98.2
45 to 54	99.1	109.7	97.0	67.7	93.1
55 to 64	102.2	101.1	101.2	118.0	105.2
65 to 74	107.0	115.9	101.9	117.2	107.0
75 and over	101.0	104.1	104.4	110.7	48.6

TABLE 3

Mutual Life Insurance Company. Cancer death rate per 100,000

AGE PERIOD	1915	1916	1917	1918	1919	1920	ALL YEARS 1915–1920
25–29	15.34	3.98	0.90	7.53	1.47	5.08	5.35
30–34	11.52	15.19	19.55	10.54	5.52	5.49	10.55
35–39	7.61	12.95	13.38	10.99	5.74	18.69	11.87
40–44	31.39	45.31	30.86	26.92	33.73	21.21	31.07
45–49	40.36	41.82	51.59	60.96	51.56	78.80	55.15
50–54	165.47	157.35	100.67	79.25	73.45	70.02	105.78
55–59	132.22	213.84	204.03	153.07	152.73	159.95	168.88
60–64	235.74	366.73	255.24	308.53	285.56	272.73	287.21
65–69	225.77	380.94	532.84	357.91	502.42	423.88	407.99
70 and over	554.09	1000.67	834.83	856.57	731.75	982.20	829.54

If the first two years, 1915–1916, combined are compared with the last two, 1919–1920, we find that up to age 65 the cancer mortality ran in general considerably lower for the later than for the earlier period, while for ages 65 and older the reverse was true. Of the nine five-year groups (including "70 and over" as such a group), beginning with age 30–34, five show a higher mortality in the earlier two-year period and four in the later. Had the subdivision into age groups been the ten-year groups of the Metropolitan, beginning with 25–34, every group up to age 65 would evidently have shown a higher mortality in the earlier period.

The year 1915, for some unexplained reason, was a year of very low cancer mortality in the Mutual, a peculiarity which did not appear in the Metropolitan experience and which was, therefore, probably accidental. It is consequently worth while to see what would have been the result had 1915 been eliminated so that we should have compared the years 1916–1917 with 1919–1920. We find that of the nine five-year groups, seven would have shown a higher mortality for the earlier period and only two for the later; moreover, had the grouping been by ten-year groups, as in the Metropolitan experience, each ten-year group from age 25 up would evidently have shown a higher mortality in the earlier period than in the later.

Again, a comparison of the last two years combined with the preceding four years combined, shows that of the nine groups five had a higher mortality in the earlier period; we must recognize, however, that two of the four were 65–69 and "70 and over" which are of greater importance than groups near the lower age limit. Had the exceptional year 1915 been eliminated, however, the result would have again looked extremely favorable for the later period.

In order to have the most recent possible data, the approximate cancer mortality for the year 1921 has been worked out, although exact figures are not yet available. The 1921 results compared with the average for the preceding six years show a lower mortality for each of the seven five-year age groups from 30–34 to 60–64, but a higher mortality for 65–69 and "70 and over."

Emphasis is given to the failure of the Mutual experience to show any tendency to an increasing death rate by the fact that the lives involved were nearly all white males, and in other experiences where the sexes are separated any tendency towards increase has generally been more among males than females.

The Metropolitan experience has been extended from 1915 through 1920 but has not yet been made public. Through the courtesy of Dr. Dublin I have had an opportunity to see these statistics and to include in this paper the deductions from them. They show in general the same lack of any tendency to increase in the age groups from age 30 to age 65, but they show an increasing tendency from age 65 upwards.

Thus, we have had the experience of the two companies covering the eleven-year period from 1911 to 1921, inclusive. The data in these experiences should probably be at least as accurate as to the causes of death as those of any other experience, because the insurance companies try to obtain accurate knowledge of the cause of death at the time the claim is paid. The figures indicate, taken at their face value without considering whether there are any modifying influences, for ages below 65 either a fluctuating or a slightly decreasing cancer mortality; for ages 65 and over they vary, but probably, on the whole, indicate some increase.

The above are the direct deductions from the statistics. Before accepting these as final results, however, we must go back of the statistics and inquire whether there are any circumstances or considerations relating to the data on which these statistics are based tending to modify the results. One such consideration should be mentioned. With the development of medical science there has been a gradual increase in correctness of diagnosis of the cause of death. Professor Walter F. Willcox (2) showed in 1917 that a very considerable apparent increase in cancer mortality would result simply from the continued improvement in the correctness of diagnosis, which increase in correctness had probably continued up to the present day. Undoubtedly in the past many deaths attributed to old age should properly have been set down as cancer, and many others attributed to other causes would undoubtedly have been attributed to cancer if the correct diagnosis had been made. The result of this would be that if there were a really stationary cancer mortality it would, nevertheless, appear to be increasing considerably because of the increasing correctness of diagnosis.

How effective this could be to cause an apparent increase where no real one exists may be seen in the statistics of appendicitis mortality which show an increase of 40 per cent from 1900 to 1915; yet undoubtedly the real rate was decreasing in this period because of surgical advance and readiness to resort to surgery.

Moreover, increasing correctness in diagnosis works almost altogether in one way; that is, it results in attributing to cancer many deaths which would formerly have been incorrectly attributed to some other cause, and in changing but few the other way. The effect of increased correctness in diagnosis, which is undoubtedly more important at the advanced ages, would seem to me enough to explain what apparent increase in cancer mortality there is in ages above 65, and it would emphasize the fact that for ages below 65 there has not been even an apparent increase.

Meanwhile the statistics of the United States Registration Area show a continuous increase in cancer deaths. These are population statistics, however, and are not analyzed as to ages; hence, as compared to such statistics as we have considered, they have very little meaning, since a change in the age proportion of the population might be responsible for the entire apparent cancer increase.

My conclusion would be, even taking into account the registration area's apparent increase, that we cannot now determine whether the cancer mortality is slightly increasing, practically stationary, or slightly decreasing, but that we can be sure it is not greatly increasing. A more exact result is something for future investigations when reliable statistics for a long period of years are obtainable.

Lest what I have said be misinterpreted I would add that such a conclusion does not lessen at all the seriousness of the cancer problem. It merely holds out hope that the terrible scourge will not increase without limit.

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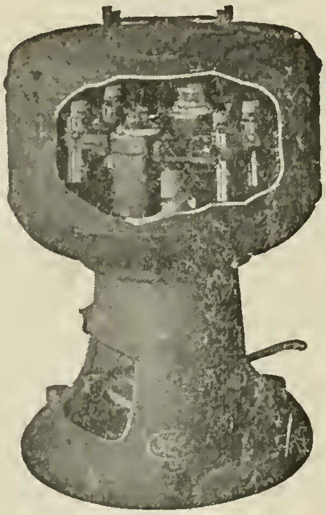

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CANCER AND PARASITE

ISIDOR KROSS[1]

From Columbia University, Institute of Cancer Research, F. C. Wood, Director

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Ever since the first recognition of the bacteria as a cause of disease, attempts have been made to demonstrate a bacterial cause for malignant tumors. The importance of such a discovery, with all its possibilities of prophylaxis or even cure, is obvious, and accounts for the sustained interest in this question, in the face of constantly repeated failure.

One of the most recent investigators is Nuzum (1) who employed a mouse carcinoma (no. 11) from the Crocker Institute. From this growth he isolated a diplococcus and described a malignant tumor having all the characteristics of carcinoma 11, which followed inoculation of a culture of this organism into mice.

The importance of such a discovery would be, needless to say, beyond computation, and it was therefore determined to repeat the experiments with additional checks and controls. That microörganisms are frequently found in malignant tumors, especially those growing in mice, is a well established fact, but, as has been said, no one has ever succeeded in proving that they play any rôle as causative agents.

To summarize Nuzum's findings, it will suffice to say that he cultivated pieces of tumor in tissue ascitic fluid media covered by paraffin, and thus partially anaerobic. From this culture he isolated a Gram-positive diplococcus, which he considers the specific bacterium in the production of carcinoma 11. A subculture was made of this organism in the same manner, and inoculated into mice. In three of them, the typical carcinoma 11 is said to have developed. However, careful perusal of the article suggests that only two of these mice had a real tumor.

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[1] Adjunct Gynecologist, Mt. Sinai Hospital, New York.

As can readily be seen, two factors must be considered in an experiment of this nature: (1) the tumor cell proper; and (2) the microörganism, if any. In order to test the ability of the latter to produce the malignant growth in question, it is necessary to prevent any participation of the tumor cells in the process. To do this without affecting the microörganism, two methods were employed in my own experiments, radiation and freezing.

In the first, several mice that had well developed carcinoma 11 tumors, were exposed to the Roentgen ray, being given a dose known to destroy the tumor cells completely. The rayed tumor was then excised aseptically and inoculated into 24 mice in the usual fashion. Part of this same tumor was cultured, following most minutely the technic laid down by Nuzum. As a control, carcinoma of the same series, from another mouse, was inoculated into 24 mice, and fragments were put at the same time into twelve tubes of the ascitic tumor fluid media. The tumor used in this control series was not radiated.

Transplantation of the unradiated control tumor resulted in the usual 60 per cent of takes which this tumor has given during the past seven or eight years at the Crocker Laboratory. On the other hand, in not a single instance did a tumor develop from the radiated cells. In other words, where the tumor-cell factor was eliminated the microörganism factor, which was being tested, was not able to reproduce the tumor.

Because of the extreme importance of the question, this experiment was repeated in another series of mice, with the same negative results.

The cultures made from the radiated tumor were incubated for five days and were then inoculated into 60 mice. In none of the 60 did a tumor subsequently develop. At the same time, smears were made from the bottom of the culture tubes and stained with Gram's and with Giemsa's stain; these contained in most instances many short and long bacilli, streptococci, staphylococci, and diplococci. In some smears all these varieties were present and most of the smears, in fact, showed more than one organism. In no instance was a pure culture of any one organism found.

The second method employed to eliminate the tumor cells was alternate freezing and thawing of the tumor before inoculation. The growth was removed aseptically and placed in a sterile dish, where it was emulsified with scissors, and the dish was then placed in an ice and salt mixture. After about fifteen minutes, when the mass was frozen solid, it was ground with a sterile pestle until it became soft and fluid. This freezing and thawing was repeated twice, with the purpose of destroying the tumor cells without harming any bacteria that might be present. Part of this emulsion was inoculated into 36 mice, and another part was cultured in the ascitic tissue fluid media.

In only two of the 36 mice was there any growth, and in both instances the tumor was not discoverable until the twenty-eighth day after inoculation, whereas in routine laboratory inoculation, growth is generally noted in from seven to ten days. Hence the freezing and thawing had killed all but a few of the tumor cells. If carcinoma 11 were due to a microörganism, all, or at least most of the mice, should have had tumors, both in the frozen and thawed tumor series, in the rayed tumor series, and in the culture inoculation series. The absence of a tumor in all but two late instances, militates against the assumption that carcinoma no. 11 can be ascribed to a microörganism, especially in view of the fact that no one type of organism was found as a constant occurrence. The two growths that did develop from frozen and ground tumor can be readily explained by the assumption that the freezing did not destroy quite all the tumor cells. Others of the staff at the Crocker Laboratory have had the same experience.

In all the cultures the results, as concerns the specific organism described by Nuzum, were negative as has been already stated and subcultures inoculated into 24 mice produced no tumors.

A careful perusal of the experiments of Nuzum leads to the conclusion that in only two instances out of eighty-nine did the culture fluid inoculation cause tumor growth, i.e., animal no.

250 in experiment 1, and a second animal in experiment 3. These two instances and our own negative findings repeatedly and carefully checked up suggest that in all probability the tumors in Nuzum's experiments were spontaneous new growths.

It may be stated in conclusion that all the animals showing negative results were kept under observation for a period of three and a half months, so that ample time was given for a tumor to arise.

CONCLUSIONS

1. While most of the ascitic tissue fluid cultures of Crocker Institute carcinoma 11 contained microörganisms of various kinds, in no instance was there found the characteristic microorganism described by Nuzum.
2. In not a single instance was the inoculation of mice with these cultures followed by tumor growth.
3. It is probable that the two undoubted tumors in Nuzum's series were a spontaneous new growth.

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TERATOMAS AND THEIR RELATION TO AGE

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The great accumulation of reported cases of teratomas offers an excellent opportunity to review the data, with the object of investigating the relation of their frequency to the age of the host. In the course of the study, it has become apparent that this relationship is so definite as to assume the form of a general law.

The largest collections of teratomas were gathered by Taruffi and Ahlfeld. Both of these observers stressed the large congenital forms which are situated in the head, the thoracic, the abdominal, or the sacral regions. Gonadal teratomas occur most frequently in early adult life (Wilms). Among others, Askanazy investigated the internal craniopagi, and Ekehorn, the internal thoracopagi. Lexer and Nakayama studied the abdominal inclusions and pygopagi.

Though many hypotheses have been advanced on the origin of teratomas, they may be resolved into two view points: The teratoma is either the offspring or the twin of its host. Stockard has recently produced experimental evidence in favor of the latter conception.

CRITERIA AND METHODS OF STUDY

Two precautions have been observed in compiling the present statistics: (1) Only growths of tridermal or bidermal origin have been considered. No such case has been omitted. (2) Special effort has been exerted to determine the age at which the teratoma began its growth. The first increase in size of external growths may be accurately observed. For those situated internally, the initial symptom was used as an indicator. Where the history was deficient, the age at which the operation took place or at which death occurred was taken as the closest approximation obtainable. The last criterium particularly applies to the teratomas of the aged. In this manner 895 cases have been studied. Sometimes a period of slow growth is followed by one of heightened activity. Such is the case of chorioma testis reported by Jackson, in which growth commenced at the age of twenty and slowly continued to twenty-three, after which the increase in size became extremely rapid. Since the relationship between the growth of the host and that of the teratoma is of interest, in such cases, the beginnings of both periods have been noted. A similar effort was made for internal teratomas, thus bringing the total number of growths tabulated to 975.

In systematizing the results it was noted that though the variation from year to year is considerable, there seems to be an orderly waxing and waning of the number of cases to an extent which justifies the drawing of a curve. An average has been drawn in order to minimize accidental variation. Six year periods have been chosen because they are the longest which correspond to actual changes throughout the length of the curve. The first period begins at fertilization and ends at five. In the curves which are drawn to a scale of one-half, the abscissae represent the age of the host when the tumor began its growth, the ordinates the number of cases in each year.

THE CURVE FOR ALL TERATOMAS

After an initial maximum rise, the curve falls and remains low from five to eleven years (fig. 1). At eleven it achieves a higher level, which is increased at seventeen and twenty-three years. The second maximum is found between twenty-three and twenty-nine years. The curve falls gradually at twenty-nine and thirty-five years, and then more rapidly at forty-one, after which it becomes progressively lower towards its end at seventy-six years. Eighty-seven per cent of the teratomas occur before forty-one years and 95 per cent before fifty-three.

Most congenital teratomas do not evince postnatal growth and since this study concerns only those which do grow, for the early maximal total may be substituted the smaller number of tumors, showing power for growth, represented by the lower broken line in the graph. With this correction the highest point in the curve is found between twenty-three and twenty-nine years. This is borne out by Wilms who finds the period of greatest frequency for sex-gland teratomas to be between the ages of twenty and thirty years. His conclusion is to be expected because teratomas occur most frequently in sex glands.

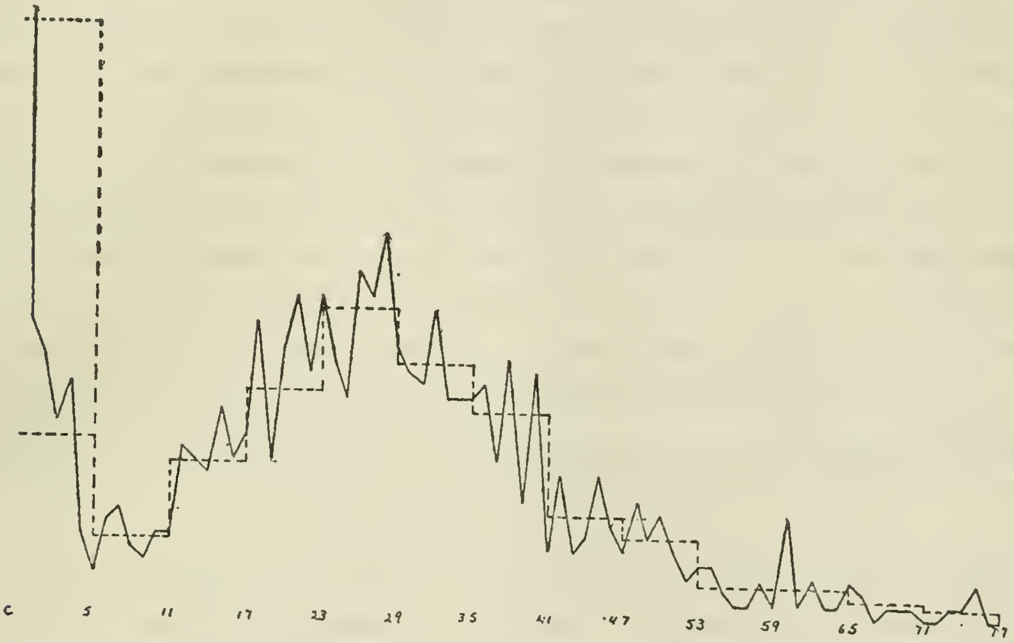

Fig. 1. Curve for all Teratomas

The abscissae stand for the years of appearance of growths, the ordinates for the number in each year. Both are drawn to a scale of one-half. The total number of congenital tumors is not shown. The dash line indicates the average for each six-year period. In the first six years there are two; the upper one stands for all cases, the lower for those which had power of postnatal growth. The mode is from twenty-three to twenty-nine, the time when growth stops.

STUDY OF THE CORRECTED CURVE

There are three aspects of the described phenomenon: First, it is evident that the total number of teratomas at any age increases with the actual growth of the individual. As size increases, the total number of teratomas increases. But it must be remembered that while size is increasing, growth rate is falling. Therefore, second, teratomas become more frequent as growth slows down, at the time when growth potential becomes smaller. Growth must be recognized as involving two elements: increasing actual proportions, and decreasing growth potential. Hence, third, the total number of teratomas at any age increases as growth potential diminishes.

When one recalls the fact that the changes in growth rate are not constant, the number of teratomas is seen to bear even a closer relationship to the growth of the hosts than has been indicated. Not only do these tumors appear as growth of the host slows, but during their time of appearance the teratomas are more frequent in the periods of slower growth of the host. Teratomas are common in early infancy following a space of the most rapid proliferation of all—fetal growth. They increase again when the comparatively rapid growth rate of early childhood gives way to the slower one of pubescence and lastly they are found in greater numbers as active growth gradually ceases.

To explain the relative number of teratomas appearing in the several periods we must take into consideration an additional factor, the growth potential of the embryonal rest, for a teratoma by most theories arises in an embryonal rest of some kind. It is known that the great majority of embryonal rests do not grow; they either degenerate or remain dormant. Others achieve a more or less perfect adult growth, while a few develop into tumors. Tridermal rests act in a similar manner. Thus we have a few with high growth potential, many with less capacity for growth, and finally others which remain latent unless they are stirred to development by an external stimulus.

During the first six years of life the growth rate of the host declines rapidly, his growth potential is greatly reduced, and the number of teratomas of relatively higher potential, capable of proliferation, is comparatively large. From five to eleven years the growth rate is fairly constant, the loss of potential is small, and the additional number of teratomas released is few. The next appreciable change takes place during the differentiation occurring at puberty and accompanying the maturation of the sex organs. Here the number of teratomas begins to increase and continues to do so until growth finally stops, at which time the greatest number of teratomas make their presence known. Although the loss of growth potential in the host becomes smaller, being least in the final period from twenty-three to twenty-nine years, yet just because growth itself is slow, there is an ever increasing number of embryonal rests of low potential, capable of expressing their latent growth energy. The very inactive rests even at this period do not have sufficient energy to start growth spontaneously and are therefore considered in another group at a later time. However, they may serve as a nidus for neoplasms since many do commence development after the growth of the host has stopped.

Thus the number of teratomas appearing at any given time depends upon the amount of loss in growth potential of the hosts during that time and the number of rests whose potential is large enough to proliferate under these conditions. Evidently *the number of teratomas in any period varies inversely to the growth potential of the hosts and directly as that of the embryonal rests.*

ANALYSIS OF CURVES OF AGE INCIDENCE OF TERATOMAS IN THE DIFFERENT LOCATIONS

The object of this study is to show the relation to the general law of the occurrence of teratomas in the various situations.

Thoracopagi. Ekehorn in his collection of teratomas of the anterior mediastinum finds fifteen cases occurring between the ages of twenty and thirty, four between thirty and forty years, and four more to sixty, thus agreeing with the present curve in showing the maximum occurrence from twenty-three years to twenty-nine.

Abdominal inclusions. The abdominal tridermal growths are frequently discovered at birth but continue to be found throughout life, with a second rise from twenty-three to twenty-nine years.

Craniopagi and pygopagi. There is not a sufficiently large number of head and sacral teratomas to yield reliable statistics.

Statistics of various authors. They show a close agreement with original statistics here presented. Note increased number of dermoids during pubescence

THORACOPAGI (CHRISTIAN)		DERMOIDS (PAULI)		EMBRYOMES (CHEVASSU)		SEMINOMES (CHEVASSU)		CARCINOMAS		
									Testis	Ovary
10–20	1	1–5	4	0–5	5	0–19	0	0–5	2	11
20–30	19	5–10	3	6–17	0	20–25	1	5–11	2	25
30–40	7	10–15	10	18–24	16	25–30	8	11–17	1	48
40–50	2	15–20	8	25–29	11	30–35	8	17–23	12	19
50 +	2	20–25	12	30–34	11	35–40	19	23–29	29	26
		25–30	14	35–39	7	40–45	14	29–35	30	21
		30–35	10	40–44	3	45–50	4	35–41	34	16
		35–40	11	45–49	4	50–60	2	41–47	27	16
		40–45	8	50–59	2	60–63	2	47–53	10	14
		45–50	10	60–75	2			53–59	8	14
		50–55	5					59–65	1	1
		55–60	3					65–71	1	
		60–65	1							
		65–70	2							
		70 +	2							
	31		103		61		59		157	207

Inclusions according to age and situation. Those which are capable of growth appear in greater number from twenty-three years to twenty-nine years

AGE	CRANIOPAGI	THORACOPAGI	ABDOMINAL PARASITES	OVARIAN TERATOMAS	TESTICULAR TERATOMAS	PYGOPAGI	TOTAL
–5	78	55	36	17	48	45	279
5–11	1	3	6	15	7	1	33
11–17	4	11	18	27	9	1	70
17–23	4	14	16	44	32	4	114
23–29	1	16	20	52	55		144
29–35		10	15	47	44		116
35–41		4	8	39	43		94
41–47		4	4	22	15		45
47–53		4	4	19	7	2	36
53–59		1	1	13			10
59–65		1	2	10	3		16
65–71			1	6	1		8
71–77	2		1	2			5
Total. . .	90	123	132	313	264	53	975

Most are congenital. The internal craniopagi occur most frequently from eleven to twenty-seven years. The only year where there is more than one case is the nineteenth, where there are two. The last teratoma capable of spontaneous growth appeared at twenty-seven. The later examples of sacral teratomas are even rarer than those occurring in the head. There is one each at ten and thirteen years and four from nineteen to twenty-three.

Ovarian teratomas. Turning next to the gonadal teratomas and comparing the testicular and ovarian curves, we see that the latter is less variable (fig. 3). This is due to the delayed diagnosis of so many of the ovarian teratomas, probably because of their slow growth, as their structure is often of the adult type. Their internal position further postpones their discovery, yet dermoids are sometimes found by accident. Nevertheless, the largest number of tumors occurs between the ages of twenty-three and twenty-nine. From a review of one hundred and three cases Pauli finds dermoids appearing most often from twenty to thirty years.

Another difference between the ovarian and testicular curves is the greater rise in the former in the two periods between eleven and twenty-three years. This phenomenon might be expected as a result of the growth differences since, in the male, postpubescent growth is more rapid than in the female.

Teratoma testis. The external situation of the male sex gland allows prompt discovery of its tumors. Although the greatest decline in rate of growth occurs early in life from birth to four years and the number of testicular teratomas at this time is large, the maximum number nevertheless occurs at a later period. This is probably due to the fact that growth under four years is comparatively very rapid in spite of its fast declining rate. The modal year of the curve is twenty-six. It is interesting to note that the last growth cartilage of the long bones ossifies at twenty-five.

Teratomas in the testes commence growth most frequently between the ages of twenty-three and twenty-nine years. Chevassu finds the maximum between twenty and thirty years (fig. 2).

TERATOMAS OF LATER LIFE

After establishing the time of greatest frequency of teratomas, it still remains to account for those of old age. Those diagnosed after the age of fifty-one may be divided into two groups. The first consists of neoplasms of adult structure which had reached the limits of their capacity for post-natal growth, while those of the second are more malignant. To the first group belong such tumors as the following:

Craniopagi. Beck reports a case in which a dermoid was found at autopsy in place of the hypophysis in a woman seventy-four years of age. Eberth reports a similar accidental finding beneath the dura in a woman of seventy-five.

Thoracopagi. There are two examples in Ekehorn's collection—Pinder's case of a patient with bulbar paralysis, aged fifty-three, in whom the dermoid was discovered at autopsy; and Lebert's of a man of sixty who had been dyspneic since his sixteenth year.

Abdominal inclusions. Rizzoli (Taruffi) reports two cases of late abdominal inclusions, one at sixty, the other at sixty-two. Symptoms had been present for a long time in both. In one of the cases they appeared first at the age of twenty.

The tumors mentioned thus far were benign, though some produced symptoms because of their size and position.

In the second group of neoplasms the element of trauma becomes important in the etiology. There is Bonney's report of a retroperitoneal chorioma of a man of sixty-seven, and Goebell's of an abdominal teratoma that became malignant at fifty-four, twenty-seven years after a mass had been diagnosed. Djewitski reports a chorioma of the bladder which first gave symptoms at the age of seventy-three. The same irritation which produces a papilloma of the bladder may transform an otherwise benign embryonal rest.

Pygopagi. Hudson describes a sacral teratoma which began growth at the age of fifty-two; the history shows that a nodule had existed in that region since the birth of the patient. The histological picture is one of a tridermal rest with cancerous degeneration of mucous glands. It is similar to an old age cancer arising in previously normal tissue. Evidently in the last four cases it is not growth potential of the embryonal rest but an extrinsic traumatic influence, to which every part of the body is subject, that caused the proliferation of cells. A case of Briddon's beautifully illustrates both these factors occurring in the same growth but independently and at different times. It concerns a sacral dermoid which appeared externally at the age of twenty-two and then ceased growth till the fifty-second year, when it underwent epitheliomatous change.

Teratoma testis. There are three examples of late teratoma testis. Lexer quotes one from v. Bergmann's clinic in a man of sixty. On section the growth was of adult structure.

Ewing and Pepere report cases which first showed growth at the ages of sixty-one and sixty-three, respectively. The microscopic examination in both instances showed carcinomatous change of one element in a totipotent rest.

Ovarian teratomas. Of thirty-two dermoids, thirteen exhibited malignant transformation of a carcinomatous, sarcomatous, or endotheliomatous type. Four showed thyroid structure, of which three were rapidly growing tumors. In six reports details were lacking. However, since the growths were called dermoids, their structure must have been of the adult type, like that of the remaining nine inclusions.

To summarize, the late appearance of stationary teratomas is due to their delayed discovery, while that of growing teratomas is caused by their injury.

Carcinoma testis. Carcinoma testis is discussed in this place not only for its possible teratomatous origin, but because traumatic etiology links it with the tumors of later life. In many cases of teratoma testis in young people the transformation of a slowly into a more rapidly growing tumor is caused by trauma. In older people the growth is rapid from the start. The same sequence of events obtains for carcinoma testis. The cell of many cases of carcinoma testis is characteristic, with a large nucleus and clear cytoplasm. Sometimes the growth is called a sarcoma; the difference in opinion is due to the fact that no analogous cell is found in the human body. This same cell is often found with teratoma testis. There are only two probable interpretations: (1) The irritation caused by some extrinsic factor, in this special case, by the teratoma on the tubule cells, is the cause of carcinoma. (2) The unique type of cell is of teratomatous origin. Chevassu takes the position that it develops from the adult spermatogonia, putting the tumor in the class of acquired carcinomas. The final convincing link in the chain of evidence has not been produced, for he has not been able to trace the steps of anaplastic change from the spermatogonia to the carcinoma cell.

It has been definitely established that the cell which is of more rapid growth will often overrun and may finally crowd out altogether the other constituents of the tumor. Thus arise the rhabdomyomas, the chondromas—the simple tumors of the sex glands. In this uncontrolled competition the most embryonal type of cell would have a decided advantage. Therefore Ewing concludes that carcinoma testis is a one-sided teratoma. In the light of the foregoing it is interesting to see to which of these two theories the carcinoma curve lends itself.

According to Chevassu's statistics embryomas occur with greatest frequency from twenty-five to thirty years and seminomas from thirty-five to forty (fig. 2). The writer's review of a larger number of cases coincides with the data of Chevassu, the modes occuring from twenty-three to twenty-nine years in the teratomas and thirty-five to forty-one years in the carcinomas.

Comparing the carcinoma testis curve with that of all cancer (Hoffman[1]), of which the congenital cases are too small a propor-

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[1] Mortality from cancer throughout the United States Registration Area. All organs and all parts. 1903–1912. (Hoffman, The Mortality from Cancer.)

	MALE	FEMALE
Until 10	1,170	984
10–24	2,028	1,844
25–34	3,757	7,891
35–44	10,750	26,779
45–54	24,431	46,669
55–64	35,327	52,393
65–74	33,745	43,010
75 and over	18,381	24,601

tion materially to alter the general outline, we see that the former has no resemblance to the latter, for in that case it would have a continuous rise to some time after sixty. In brief, the carcinoma testis curve is the teratoma testis curve with the mode slightly shifted.

Since the histogenesis of carcinoma testis has not been traced from either embryonal or adult tubule cells, it is probable that carcinoma testis is of nontesticular origin, and since there is no reason why misplaced cells should so often be of the same type or occur so frequently with teratomas, unless they are of teratomatous origin, we are forced to this conclusion towards which the study of the curve gives additional evidence. Similarly to teratoma testis, carcinoma may show a congenital increase in the size of the organ. Morestin (Chevassu) reports such a tumor, which assumed malignancy at the age of thirty-seven. Like-

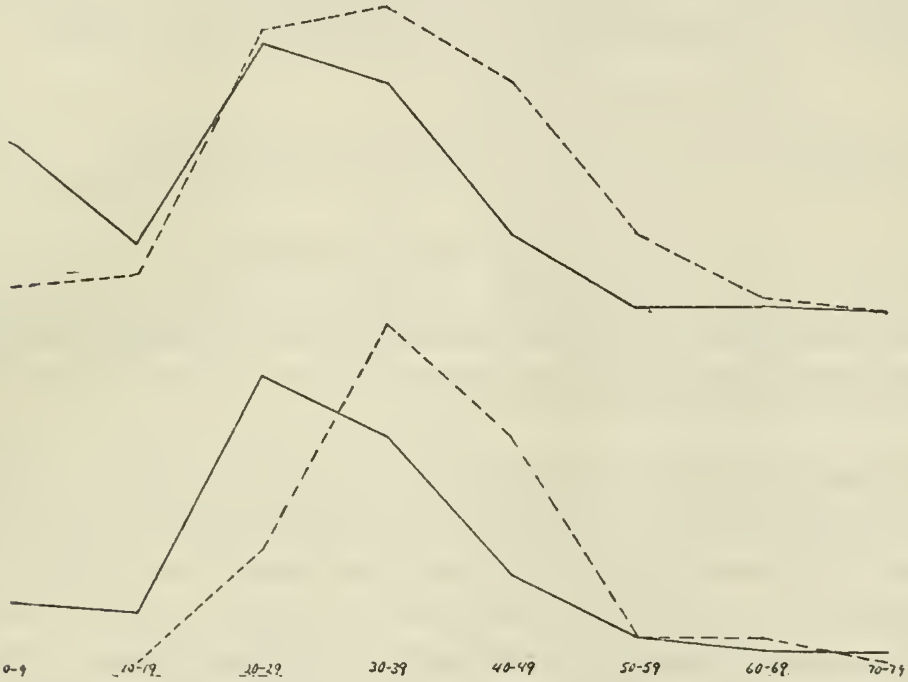

FIG. 2. TESTICULAR TERATOMAS

The lower two curves are reproduced from Chevassu's paper. The continuous line represents the teratomas; the dash line, the carcinomas. In order better to compare the new curves with those of Chevassu, the number of teratomas in each year was divided by four, and that of carcinomas by two. Both curves are plotted on a basis of ten-year periods.

wise carcinoma occurs more often in undescended testicles. It begins its growth in the rete and, like teratoma testis, is occasionally observed in pseudohermaphrodites.

Ovarian tumors. The origin of primary tumors of the ovary is so undecided that any data in reference to them is of particular interest. Here we shall mention a few facts in regard to one of these tumors which may be of teratomatous origin, i.e., the sarcoma.

Ewing divides these sarcomas into three main types: (1) spindle cell; (2) round cell; (3) myxoma cell. This classification is of special significance since just such types of sarcomatous degeneration of dermoids have been observed (Debucy).

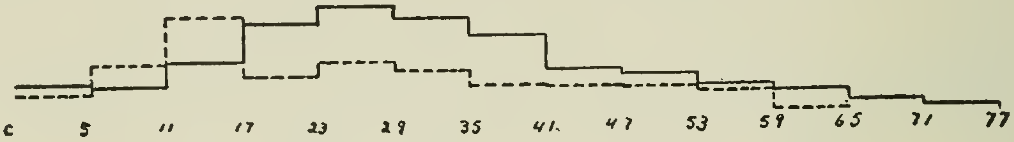

Fig. 3. Ovarian Teratomas

These curves are averages for six year periods drawn to a scale of one-half. The continuous line represents the teratomas with the mode from twenty-three to twenty-nine years; the dash line the carcinomas with the mode from eleven to seventeen years, the period of pubescence.

Desurmont finds that the different primary tumors are bilateral to varying degrees. However, sarcomas, 25 per cent, and dermoids, 20 per cent (Pauli), approximate each other quite closely.

Finally, the most common tumor of infancy is the sarcoma, which is most frequent at fifteen years (Donhauser). Cordier and Zangemeister give fifteen and twenty years, respectively, as the age of most common occurrence of sarcomas. They are found from fifteen years to twenty-five and from forty years to fifty (Desurmont), both periods of physiological stimulation. The writer finds the mode of the combined carcinoma and sarcoma curve at fifteen years (fig. 3). Comparing the mode of this curve with that of ovarian teratomas, we find that it has been shifted forward to the time of pubescence. Hence there is a group of embryonal cell tumors having an age incidence similar to teratomas, and becoming malignant under the stimulation of puberty.

FACTORS IN THE ETIOLOGY OF TERATOMAS

The growth of the earlier teratomas may be adequately explained on the basis of a growth competition between the host and the embryonal rest. But even in the teratomas of infancy another factor, trauma, may be present. It becomes increasingly important later on.

Growth potential. The growth of the host inhibits that of the teratoma.

The growth of the embryonal rest may be divided into two parts: its prenatal development, or growth which continues until stopped by the inhibition produced by the excessive growth of the host; and the growth of which it is still capable (growth potential) after that of the host slows down or ceases. These two parts are in reciprocal relation to each other. The earlier the prenatal inhibition, the smaller and less differentiated will be the inhibited rest but the greater will be the remaining growth potential. Small embryonal rests may develop proliferative powers while large ones, which achieve a certain intrauterine development, seldom if ever show further capacity for spontaneous growth.

Trauma. The shift in the mode of the curve of carcinoma testis from twenty-three to twenty-nine years to thirty-five to forty-one years is due to an external stimulus.

In spite of the larger growth potential of the smaller testicular rests many do not achieve malignancy until their immediate region is traumatized. This is illustrated on comparing these tumors in the testicle and the ovary. A larger proportion of the latter are benign, forming adult structures. The chief difference in their histories is due to their locations; the testicle is exposed to injury, the ovary is not. It is generally admitted that teratomas do not become malignant much oftener than do normal tissues.

In the case of carcinoma testis we should expect with a history of injury to a small undifferentiated rest and the resulting proliferation of an embryonal cell, a shift in the mode of the curve towards that of old age cancer.

SUMMARY AND CONCLUSIONS

1. *Growth potential and teratomas*

1. Parasite. Teratomas are tridermal embryonal rests endowed with a certain amount of possible growth, i.e., growth potential. When the rest is comparatively large it has necessarily consumed considerable growth energy before birth, while in the small teratoma, the growth period may be divided into two parts, a slight early growth soon followed by an inhibition, and a later, or post-natal growth, should conditions permit.

2. Host. The total number of teratomas in a population, up to any given age, increases while growth potential of the hosts decreases. As the larger increases in the number of teratomas occur in periods when growth of the host is slowed most, the growth of the host must inhibit that of the embryonal rest.

3. The number of teratomas appearing in any given time varies inversely with the growth potential of the host and directly as that of the embryonal rest. The tumors which begin their postnatal growth before that of the host stops are of highest potential, but are not necessarily more malignant, for they must overcome a still present inhibition. Since most teratomas have a low growth potential, they appear most commonly at the time the growth of the host stops—from twenty-three to twenty-nine years.

II. *Trauma and autonomous growths*

1. Teratomas which start growth as a result of injury are malignant more frequently than those which proliferate solely under the influence of growth potential.

2. When trauma precipitates growth, the teratoma is frequently monodermal. If the inclusion is still in an undifferentiated condition the cell is often of an embryonal type. If a developed inclusion is traumatized the cell in many instances is like that of acquired cancer.

3. The curve of carcinoma testis rises and falls in a manner similar to that of teratoma testis and not like that of old age

cancer. This is another fact which may be adduced in support of the theory that carcinoma testis is a one-sided teratoma. In the female, a similar neoplasm might be expected to arise as the result of the physiologic stimulations of puberty. This is what actually takes place, hence the growth, in all probability, is of teratomatous origin.

4. Trauma is followed by proliferation of cells, and any precipitant of regeneration may be important in the etiology of acquired cancer. In the old the inhibition of the organism is almost negligible. Hence trauma at that time may readily be followed by an uncontrolled and therefore excessive growth. Thus, loss of growth restraint may be almost as important a factor in the etiology of acquired cancer as in that of congenital inclusions.

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[2] These references were chosen out of over one hundred and sixty; they contain extensive reviews of the literature consulted.

AN ATYPICAL ADENOMA OF THE PANCREAS ORIGINATING IN ISLET TISSUE

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True simple adenomata of the pancreas arising from the islands of Langerhans have been described by several authors. The condition is very rare and the diagnosis is somewhat difficult to establish. The tumors hitherto described have been very small, the largest being 11 mm. in diameter. Most of them were accidental autopsy findings, and some were discovered only in the sections prepared for routine microscopical examination. Nichols (10), in 1902, was one of the first to report such a tumor. He stated clearly and convincingly upon what grounds he based the diagnosis, and subsequent authors seem to have guided themselves according to those facts in deciding the islet origin of an adenoma of the pancreas as opposed to the other two possible sources, viz., duct and adult acini. Helmholz (3) in 1907, Morse (8) in 1908, and Koch (6) in 1914, described cases which fulfilled the requirements stated by Nichols and concluded that they were dealing with a simple adenoma originating in the islands of Langerhans. LeComte (7) in 1913, described a case of his own and also reviewed the literature to that date. He determined that adenomata of the pancreas have no great clinical significance and "constitute no very serious hindrance to long life." He classified as true adenomata of the islands those reported by Nichols (10), Ssobolew (13), Reitmann (11), Herxheimer (4), Helmholz (3), Morse (8), Cecil (1), Weichselbaum (14), Rollet (12), and Heiberg (2). Although he favors a diagnosis of adenoma in the above cases, some of the authors themselves, notably Ssobolew, Herxheimer, Weichselbaum, and

Cecil, either consider the nodules to be hypertrophic islands, or do not decide definitely between a diagnosis of simple hypertrophy and neoplasm. Whenever there is doubt between the diagnosis of simple hypertrophy and new growth he recommends the latter if the nodule exceeds the arbitrary diameter of 1 mm., provided, of course, that it shows all the characteristics of island tissue. Others have reported the same lesion and several have gone so far as to trace the origin of some carcinomata of the pancreas to the islands. The conclusions of some of these authors, especially of Fabozzi, who reported five cases, have been disputed vigorously. With the exception of Horgan (5) most of these investigators studied advanced carcinomata so that a doubt of validity of their conclusions seems justifiable.

The tumor of the pancreas to be described here was discovered at autopsy by Dr. H. T. Karsner, performed through the courtesy of Dr. John Maclachlan.

SUMMARY OF CLINICAL HISTORY AND PHYSICAL EXAMINATION

The patient is an unmarried white female fifty-five years of age, in whose past history nothing of importance is noted save that eight years ago a myomectomy was performed with uncomplicated recovery. Two years ago she noticed looseness of the bowels, which over several months progressively became worse and ultimately became a profound diarrhea. This was accompanied by gradual loss of flesh and strength but she was not confined to bed until about two months before death. In the last weeks of her life bowel movements were extremely frequent but well controlled, sometimes numbering 16 to 20 in the course of twenty-four hours. No microscopic or chemical examination of the stools was made but the gross examination showed thin watery stools with very little mucus and no blood. At various times indigestion of the different food elements was noted. On a milk diet the stools, upon standing, showed a supernatant layer of undigested fat. When meat was introduced into the diet, undigested fragments were seen in the stools. Fruits such as banana were easily recognizable. Fermention was never marked nor was putrefaction severe. The clinical examination showed no abnormality of lungs, heart, abdomen, or nervous system. The patient finally died apparently as the result of profound asthenia.

SUMMARY OF AUTOPSY FINDINGS

The body is that of a white female apparently fifty-five years of age, showing moderate emaciation. Upon examination of the internal organs there were found, in addition to the tumor of the pancreas described in detail below, slight chronic interstitial nephritis, fatty metamorphosis of the liver, subacute entero-colitis, and slight passive congestion of lungs, liver, kidneys, and intestines.

SPECIAL EXAMINATION

Gross description

When the abdomen is opened the pancreas is found in the normal location, but a tumor mass partly embedded in the head presents above the lesser curvature of the stomach. This tumor is not adherent to any of the structures in the neighborhood. The pancreas measures 15 cm. in length. Partly embedded in the head of it, 10 cm. from the tip of the tail, is a mass, roughly spherical in shape and measuring 4.5 by 3.5 by 2.5 cm. This tumor is definitely and completely encapsulated so that it can be enucleated easily from the head of the pancreas. The capsule is thin, pearly white in color, and in it, coursing over the surface of the growth, are several dilated vessels, apparently veins. The tumor is firm but elastic and cuts with moderate resistance. The cut surface bulges moderately, is light yellow in color, and bleeds slightly. There is moderate vascularization of the tissue but no areas of hemorrhage are seen. Near the center of the mass there is one small area of necrosis about 3 mm. in diameter. The central portion of the growth is of a slightly deeper yellow color, is somewhat firmer, and bulges to a greater extent than the tissue at the periphery. The tissue is only moderately friable. The head of the pancreas is normal save for moderate congestion. The entire portion of the pancreas from the tumor to the tip of the tail is the seat of marked atrophy. In this portion the cross section measures 12 by 5 mm. The lobules are obviously reduced in size, there is slight increase of the interlobular connective tissue, and a moderate amount of fat infiltration.

HISTOLOGICAL DESCRIPTION

Sections were stained by hematoxylin and eosin, eosin methylene blue, thionin, and Mallory's connective tissue stain.

Pancreas

The duodenal portion shows very advanced autolytic changes, so advanced, in fact that little description is justified beyond noting a slight increase in connective tissue both between and within the lobules. Several definite islets are found which are of normal size and structure. The tail shows great reduction in the size of the lobules, acini, and individual cells comprising them. There is a great increase of the interlobular as well as interacinar connective tissue, and in it are a few foci of lymphoid cells. The smaller ducts are well preserved and normal, but the larger ducts show desquamation of the lining epithelium in some instances and complete digestion in others. The islets of Langerhans are moderately abundant, of normal size and structure, and fairly well preserved. There is no sign of invasion of the pancreatic tissue by new growth or of neoplastic change in the cells of the pancreas.

Tumor

There is a definite but thin capsule which consists of dense, moderately nucleated fibrous connective tissue. This shows no sign of invasion by the parenchyma of the tumor. Throughout the tumor there are present anastomosing bands of connective tissue, some of which are densely fibrous and some rather loosely arranged. Many of these trabeculae do not join at all and are seen as isolated masses of connective tissue which vary considerably in size and in shape, some being elliptical or rounded, and others stellate or irregularly branching. Nearly every one of them contains one or more blood-vessels. Some of these vessels are very thin-walled, consisting apparently of only a single layer of endothelial cells, while others are large and well formed with a thick wall consisting of intima, media, and adventitia. Most of the vessels are filled with blood. In some of the trabeculae there are seen merely blood spaces showing no definite endothelial lining. The trabeculae give to the parenchyma of the tumor an alveolated appearance. The parenchyma consists of numerous masses and anastomosing bands and strands of epithelial cells. These masses vary greatly in size and shape. Some are in intimate contact with the bands of connective tissue described above, but in most instances they are retracted from them. This is very likely due to fixation. Between the individual epithelial cells there is no reticulum. Cell outline in many areas is rather indistinct, but for the most part the cells are polyhedral or rounded in shape. Most of the cells are arranged in the form of solid masses, but some of the latter are hollowed out and in a few sections apparent attempts at acinar arrangement are seen, the cells surrounding these imperfectly

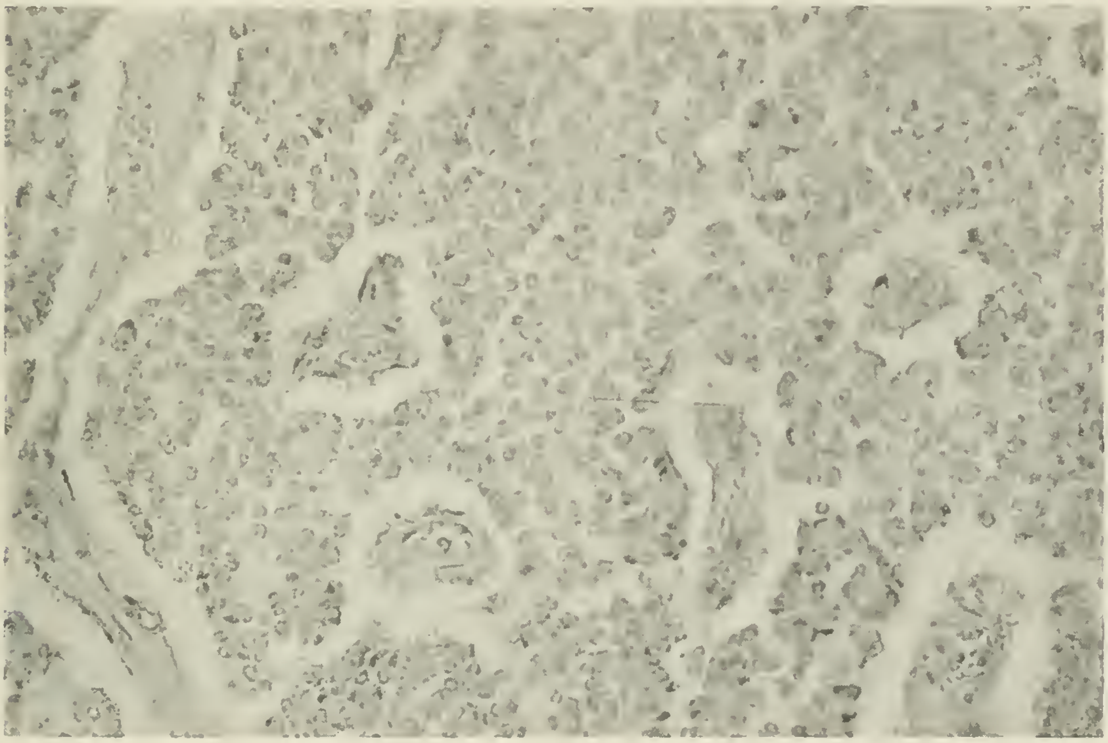

Fig. 1. Photomicrograph Showing Type of Cell, Arrangement of Cells and the Supporting Connective Tissue Containing Capillaries

formed acini being of the cylindrical type. In many sections, what at first appear to be fairly well-formed acini are seen to contain in their lumen small masses of loosely arranged moderately nucleated connective tissue. These are formed apparently by the ensnaring of some of the connective tissue stroma by strands of epithelial cells which happen to be arranged in a circular manner. Many of these small areas of connective tissue within the pseudo-acini contain one or more small capillaries. Wherever the connective tissue is small in amount the epithelial cells come into such intimate contact with the capillaries that a spinous peritheliomatous appearance is the result. It is impossible to identify a well formed typical acinus, but in a few cases, owing to the cylindrical appearance of the cells and the pseudo-acinar arrangement one cannot rule out the possibility that this represents a feeble attempt at the formation of a tubule or gland follicle.

Most of the tumor cells are polyhedral or rounded in shape, are approximately of the size of islet cells, in most instances definitely smaller than those of pancreatic acini, and show the same variation in size that the islet cells do. An occasional very large cell is seen but this is not uncommon in the normal islet. The cytoplasm of the cells is relatively moderate in amount, is definitely acidophilic, stains very lightly, and is granular. As in the nuclei of the islet cells, the chromatin is aggregated into a single large mass or several, usually three or four, small clumps. The examination of very many sections failed to reveal a single mitotic figure.

The general architecture of the tumor as described above bears a very striking resemblance to that of the islets of Langerhans, so much so that if certain portions in which the masses and cords of epithelial cells are not large were seen in a normal pancreas they would fulfill all the morphological requirements of an island. A careful study of the cells themselves confirms this very striking resemblance.

SUMMARY

That this tumor is of benign nature is deduced from the following facts: It is definitely and completely encapsulated.

Neither the capsule nor the neighboring pancreatic tissue shows any signs of direct invasion by the new growth. There is no metastasis. There are no mitotic figures. The general arrangement of the tumor is not unlike that of some cellular adenomata found in other organs of the body. The epithelial cells of the tumor and their arrangement with relation to the vascularized trabeculae show unmistakable signs of differentiation, since there is such a striking resemblance to the islets of Langerhans. In size, shape, staining characteristics, and the minute structure of the cytoplasm and nucleus the cells resemble very closely those of the islands.

The tumor here described is in all respects like those reported by Nichols, Helmholz, Morse, and others. The only differences are that this growth is much larger than any hitherto reported, and that by reason of its size and location it had exerted pressure upon the pancreatic ducts and had caused clinical signs and symptoms suggestive of pancreatic insufficiency. In this respect it differed from most adenomata hitherto described. Glycosuria was never found.

CONCLUSION

An adenoma of the head of the pancreas has been described which originated in an islet of Langerhans.

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THE INFLUENCE UPON THE GROWTH OF TRANSPLANTED FLEXNER-JOBLING RAT CARCINOMA OF HYDROGEN IONS AND OF VARIOUS SALTS IN DIFFERENT CONCENTRATIONS

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1. INTRODUCTION

In connection with the investigation of certain enzyme actions of extracts of malignant human and rat tumors, the results of which are published elsewhere (1), a study was made of the effects upon their subsequent growth of various treatments of the Flexner-Jobling rat carcinoma transplants before inoculation. The results presented here include the éffects of solutions of different hydrogen ion concentrations and of some simple salts and their mixtures.

2. EXPERIMENTAL METHODS AND RESULTS

a. Tumor transplantation method

Albino rats, both male and female, were used in this work. They were inoculated with the Flexner-Jobling rat carcinoma in the usual way, and the tumors were allowed to grow for a period of from six to seven weeks (2). The term $\frac{\text{FRC},}{\text{86A}}$ for example, indicates that the tumor was derived from the 86th generation, series A. The rats were fed on wheat bread soaked in whole milk, fresh cabbage or carrots, and fresh tap water *ad libitum*.

In carrying out the transplantations, six (or more) small particles of tumor tissue each weighing about 5 mgm., and one piece weighing about 50 mgm., were selected from the non-necrotic area of the rat tumor and placed in 25-cc. portions of solutions of definite hydrogen ion concentration and salt content previously sterilized in a steam autoclave at fifteen pounds pressure for fifteen minutes. The flasks containing the solutions and tumor fragments were allowed to remain in the ice box for definite intervals of time of from one-half to seventy-two hours. At the end of this period of time, the smaller pieces of tumor tissue were inoculated with a trochar into rats subcutaneously in the region of the right axilla. At the same time, the larger as well as some of the smaller pieces of tumor tissue were fixed in formaldehyde solution for histological examination. As a control, the same number of animals were inoculated with untreated tumor tissue immediately after its removal from the tumor-bearing animal. The progress of the transplanted tumors in the rats was recorded graphically by measuring them every week by means of calipers.

b. Preparation of solutions

The pH values of the solutions were determined in most cases by means of indicators and the standard solutions recommended by Clark (3), and in some potentiometrically. The salts used were purified by crystallization in the usual manner whenever necessary. The compositions of the various solutions in which the tumor fragments were immersed are given in table 1.

c. Growth of transplanted tumors after different treatments

The complete results of the transplantation experiments are recorded in 39 charts. As it is manifestly impracticable, even if desirable, to reproduce this number of charts, the results will be given in the first instance in table 1 as far as possible. Although this method of presentation leaves much to be desired, especially in the recording of the detailed growths of the various transplants, it will serve to bring out the points which are to be emphasized. In order to present a partial idea, at any rate, of the various growths, a number of more or less typical examples were chosen from the charts and are recorded in figure 1.

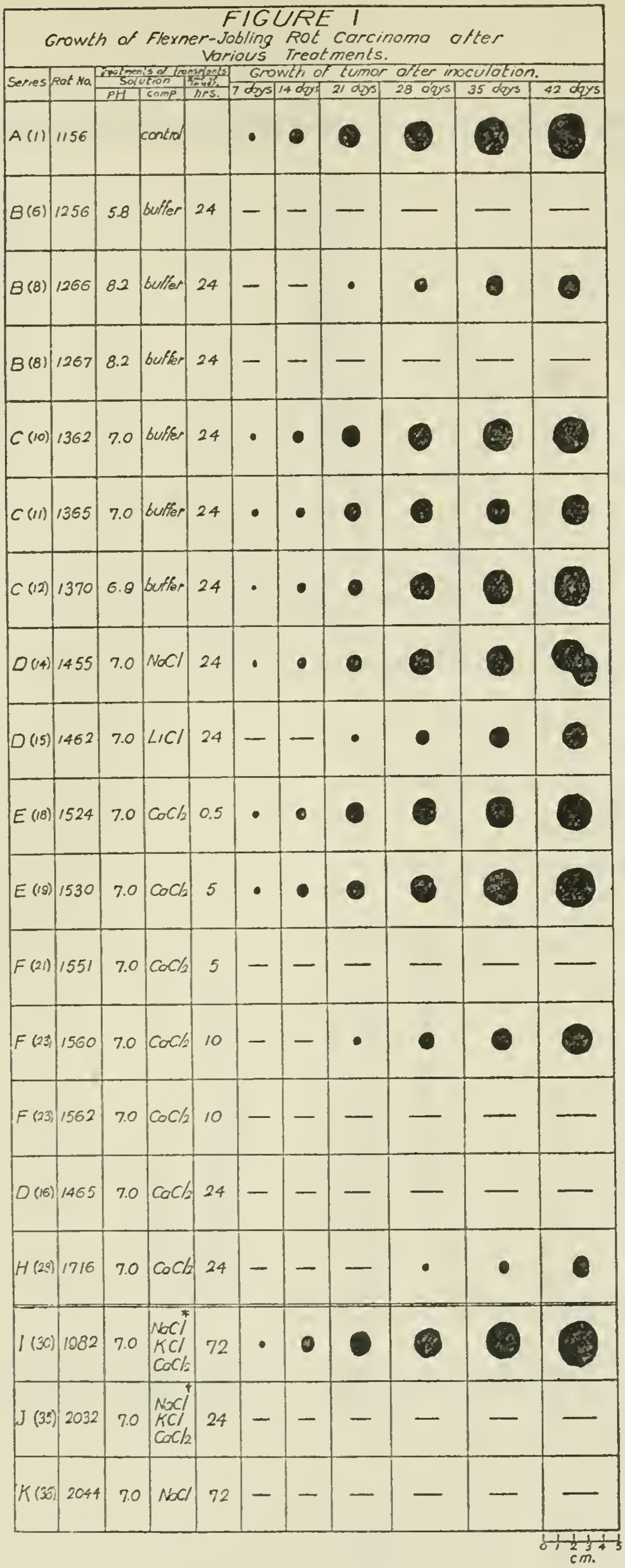

FIGURE I

Growth of Flexner-Jobling Rat Carcinoma after Various Treatments.

Series	Rat No.	Treatments of transplants Solution			Growth of tumor after inoculation.					
		PH	comp	[illegible] hrs.	7 days	14 days	21 days	28 days	35 days	42 days
A (1)	1156		control		●	●	●	●	●	●
B (6)	1256	5.8	buffer	24	—	—	—	—	—	—
B (8)	1266	8.2	buffer	24	—	—	●	●	●	●
B (8)	1267	8.2	buffer	24	—	—	—	—	—	—
C (10)	1362	7.0	buffer	24	●	●	●	●	●	●
C (11)	1365	7.0	buffer	24	●	●	●	●	●	●
C (12)	1370	6.9	buffer	24	●	●	●	●	●	●
D (14)	1455	7.0	NaCl	24	●	●	●	●	●	●
D (15)	1462	7.0	LiCl	24	—	—	●	●	●	●
E (18)	1524	7.0	$CaCl_2$	0.5	●	●	●	●	●	●
E (19)	1530	7.0	$CaCl_2$	5	●	●	●	●	●	●
F (21)	1551	7.0	$CaCl_2$	5	—	—	—	—	—	—
F (23)	1560	7.0	$CaCl_2$	10	—	—	●	●	●	●
F (23)	1562	7.0	$CaCl_2$	10	—	—	—	—	—	—
D (16)	1465	7.0	$CaCl_2$	24	—	—	—	—	—	—
H (29)	1716	7.0	$CaCl_2$	24	—	—	—	●	●	●
I (30)	1082	7.0	NaCl* KCl $CaCl_2$	72	●	●	●	●	●	●
J (35)	2032	7.0	NaCl† KCl $CaCl_2$	24	—	—	—	—	—	—
K (36)	2044	7.0	NaCl	72	—	—	—	—	—	—

Fig. 1. Growth of transplanted Flexner-Jobling rat carcinoma after immersion in various salt solutions and in solutions of different hydrogen ion concentrations.

* Concentration of salts as in Locke-Ringer solution.

† Concentrations of salts three times those in Locke-Ringer solution.

TABLE 1

Results of transplanting Flexner-Jobling rat carcinoma after various treatments

SERIES	NUMBER OF ANIMALS USED	TUMOR USED	IMMERSION OF TUMOR FRAGMENTS BEFORE INOCULATION			GROWTH OF TRANSPLANTS	REMARKS
			Composition of solutions	pH	Time of immersion		
					hours	*per cent*	
A(1)	5	$\frac{\text{FRC}}{\text{90B}}$	Controls			100	Rapid growth
A(2)	5	$\frac{\text{FRC}}{\text{90B}}$	50 cc. M/5 KH_2PO_4 + 1 cc. M/5 KOH, diluted to 200 cc.	5.1	24	0	No growth
A(3)	5	$\frac{\text{FRC}}{\text{90B}}$	50 cc. M/5 KH_2PO_4 + 29.6 cc. M/5 KOH, dil. to 200 cc.	7.0	24	75	One rat died shortly after the inoculation, one failed to develop the tumor, others grew normally
A(4)	5	$\frac{\text{FRC}}{\text{90B}}$	50 cc. M/5 KH_2PO_4 + 53.0 cc. M/5 KOH, dil. to 200 cc.	8.8	24	0	No growth
B(5)	5	$\frac{\text{FRC}}{\text{91A}}$	Controls			100	Rapid growth
B(6)	5	$\frac{\text{FRC}}{\text{91A}}$	50 cc. M/5 KH_2PO_4 + 5.7 cc. M/5 KOH, dil. to 200 cc.	5.8	24	0	No growth
B(7)	5	$\frac{\text{FRC}}{\text{91A}}$	50 cc. M/5 KH_2PO_4 + 29.6 cc. M/5 KOH, dil. to 200 cc.	7.0	24	(100)	With two rats, no growth in first week, normal thereafter; other two, normal growth at once; one rat, growth after second week

B(8)	5	$\frac{\text{FRC}}{\text{91A}}$	50 cc. M /5 KH_2PO_4 + 51.0 cc. M /5 KOH, dil. to 200 cc.	8.2	24	(60)	With three rats, no growth for two weeks, then normal growth; with two rats, no growth at all
C(9)	5	$\frac{\text{FRC}}{\text{91C}}$	Controls			80	Four grew rapidly, one retrogressed after second week and disappeared in fifth week
C10)	5	$\frac{\text{FRC}}{\text{91C}}$	35 cc. M /5 KH_2PO_4 + 20.8 cc. M /5 KOH, dil. to 200 cc.	7.0	24	100	One tumor developed slowly, rest grew rapidly
C(11)	5	$\frac{\text{FRC}}{\text{91C}}$	50 cc. M /5 KH_2PO_4 + 29.6 cc. M /5 KOH, dil. to 200 cc.	7.0	24	100	With two, no growth in first week, normal thereafter; other three, normal growth at once
C(12)	5	$\frac{\text{FRC}}{\text{91C}}$	65 cc. M /5 KH_2PO_4 + 39.0 cc. M /5 KOH, dil. to 200 cc.	6.9	24	100	With two, no growth in first week, normal thereafter; other three, normal growth at once
D(13)	5	$\frac{\text{FRC}}{\text{92A}}$	Controls			100	Four grew rapidly, one began to retrogress after fourth week
D(14)	5	$\frac{\text{FRC}}{\text{92A}}$	0.15 M NaCl (0.88 per cent)	7.0	24	100	Slow growth during first week, rapid thereafter
D(15)	5	$\frac{\text{FRC}}{\text{92A}}$	0.15 M LiCl (0.64 per cent)	7.0	24	60	Two rats, no tumor growth; two rats, normal growth; one normal after second week

TABLE 1—*Continued*

SERIES	NUMBER OF ANIMALS USED	TUMOR USED	IMMERSION OF TUMOR FRAGMENTS BEFORE INOCULATION			GROWTH OF TRANSPLANTS	REMARKS
			Composition of solutions	pH	Time of immersion		
					hours	*per cent*	
D(16)	5	$\frac{\text{FRC}}{\text{92A}}$	0.078 M $CaCl_2$	7.0	24	0	No growth
E(17)	5	$\frac{\text{FRC}}{\text{94A}}$	Controls			100	Rapid growth
E(18)	5	$\frac{\text{FRC}}{\text{94A}}$	0.078 M $CaCl_2$	7.0	½	100	Rapid growth
E(19)	5	$\frac{\text{FRC}}{\text{94A}}$	0.078 M $CaCl_2$	7.0	5	80	Four grew normally; one, no growth
F(20)	5	$\frac{\text{FRC}}{\text{94B}}$	Controls			100	Rapid growth
F(21)	5	$\frac{\text{FRC}}{\text{94B}}$	0.078 M $CaCl_2$	7.0	5	20	One, normal growth; four, no growth
F(22)	5	$\frac{\text{FRC}}{\text{94B}}$	50 cc. M /5 KH_2PO_4 + 5.7 cc. M /5 KOH, dil. to 200 cc.	5.8	5	100	Normal growth
F(23)	5	$\frac{\text{FRC}}{\text{94B}}$	0.078 M $CaCl_2$	7.0	10	(40)	Three, no growth; one, normal growth after second week; one, normal growth after third week

F(24)	5	$\frac{\text{FRC}}{\text{94B}}$	0.078 M $CaCl_2$	7.0	24	(80)	One, no growth; two, normal growth after second week; two, normal growth after third week
G(25)	5	$\frac{\text{FRC}}{\text{96A}}$	Controls			80	Four, normal growth, one rat died
G(26)	8	$\frac{\text{FRC}}{\text{96A}}$	0.078 M $CaCl_2$	7.0	24	0	No growth
H(27)	6	$\frac{\text{FRC}}{\text{97A}}$	Controls			83	Five, normal growth; one tumor retrogressed after second week
H(28)	10	$\frac{\text{FRC}}{\text{97A}}$	0.078 M $CaCl_2$	7.0	24	(70)	Three, no growth; four grew normally after second week; three grew normally after third week
I(29)	10	$\frac{\text{FRC}}{\text{99B}}$	Controls			100	One tumor grew slowly; rest rapidly
I(30)	5	$\frac{\text{FRC}}{\text{99B}}$	0.15 M NaCl 0.003 M KCl 0.003 M $CaCl_2$	7.0	72	80	One tumor did not grow; others grew normally
I(31)	5	$\frac{\text{FRC}}{\text{99B}}$	0.45 M NaCl 0.009 M KCl 0.009 M $CaCl_2$	7.0	72	0	No growth
I(32)	5	$\frac{\text{FRC}}{\text{99B}}$	0.45 M NaCl	7.0	72	0	No growth

TABLE 1—*Concluded*

SERIES	NUMBER OF ANIMALS USED	TUMOR USED	IMMERSION OF TUMOR FRAGMENTS BEFORE INOCULATION			GROWTH OF TRANSPLANTS	REMARKS
			Composition of solutions	pH	Time of immersion		
					hours	*per cent*	
I(33)	5	$\frac{\text{FRC}}{\text{99B}}$	0.009 M $CaCl_2$	7.0	72	0	No growth
J(34)	10	$\frac{\text{FRC}}{\text{100B}}$	Controls			80	One tumor retrogressed; one did not grow; rest grew normally
J(35)	5	$\frac{\text{FRC}}{\text{100B}}$	0.45 M NaCl 0.009 M KCl 0.009 M $CaCl_2$	7.0	24	0	No growth
J(36)	5	$\frac{\text{FRC}}{\text{100B}}$	0.009 M $CaCl_2$	7.0	24	0	No growth
K(37)	14	$\frac{\text{FRC}}{\text{100C}}$	Controls			93	One tumor retrogressed; rest grew normally
K(38)	5	$\frac{\text{FRC}}{\text{100C}}$	0.15 M NaCl	7.0	72	0	No growth
K(39)	5	$\frac{\text{FRC}}{\text{100C}}$	0.003 M $CaCl_2$	7.0	72	0	No growth

The data given in table 1 are perhaps sufficiently self-explanatory. The experiments were allowed to proceed six to seven weeks in every case. In the column showing percentage of positive inoculations, whenever the growth was delayed for a period greater than one week, it was calculated as a positive inoculation, but the number indicating the percentage was enclosed in parentheses. Figure 1 shows the relative growths under the different conditions more clearly.

The results of the various treatments on the growth of the transplants can be summarized in a comparatively brief manner as follows:

1. After immersion in potassium phosphate buffer mixtures of various hydrogen ion concentration for twenty-four hours, the following results were obtained: No effect was observable at pH 7.0, the growths being practically the same as in the control; no growths at all were obtained at pH 5.8 and 5.1; at pH 8.2 partial inhibition and delayed growths were found; and no growths at pH 8.8.
2. Varying the concentration of the buffer mixture (from approximately 0.6 per cent to 1.1 per cent) at pH 7.0 (series C10, C11, and C12) and immersing for twenty-four hours did not produce any effect, the growths being normal in all the cases.
3. Sodium chloride (0.15 M or 0.9 per cent) at pH 7.0 had no effect in twenty-four hours on the growth of the transplants; seventy-two hours immersion resulted in complete inhibition of the growths; lithium chloride at pH 7.0 in twenty-four hours caused partial inhibition.
4. Calcium chloride at pH 7.0 inhibited or retarded the growths of the transplants. Immersion for one-half hour had no effect; for five and ten hours, partial inhibition or retardation; for twenty-four hours and longer, complete inhibition.
5. Immersion in a Locke-Ringer solution for seventy-two hours did not affect the growths. Immersion in a solution containing three times the concentration of the sodium, calcium, and potassium chlorides in the Locke-Ringer solution for twenty-four or seventy-two hours resulted in complete inhibition of the tumor transplants.

d. Histological examinations of tumors

The histological examinations of a number of the tumor transplants after immersion in the various solutions were made by Dr. James Ewing, to whom the writers wish to express their thanks. The general structure after immersion in the phosphate mixtures of various hydrogen ion concentrations showed slight hydropic degeneration in the case of pH 7.0, but with the more acid and more alkaline solutions the tumor cells had undergone marked hydropic degeneration, being larger and considerably swollen. The microscopical structure of this tumor is shown in plates 1 and 2; in plate 1 an untreated section, and in plate 2 one treated with a solution of pH 5.8. The histological comparison of untreated tumor and tissue which had been immersed in solutions of sodium chloride, lithium chloride, and calcium chloride also showed some interesting facts. In those treated with sodium or lithium chloride, there was considerable accumulation of fluid between the tumor cells and many nuclei appeared slightly shrunken. On the other hand, tissue which had been treated with calcium chloride showed imbibition of fluid in the intercellular spaces and the cells themselves were shrunken. The nuclei stained more densely and the chromatin was condensed to a central mass within the nuclei. The cytoplasm showed hydropic vacuolization (plate 3). A microscopical examination of tumor tissues was made after immersion for seventy-two hours in a Locke-Ringer solution, and also of the same tumor tissue treated by a modified Locke-Ringer solution in which the salt content was increased three times. Under both conditions the tissues showed marked hydropic degeneration, but the proliferating capacity of the tissue treated with normal Locke-Ringer solution was not altered; while under the second condition proliferation was completely inhibited.

3. DISCUSSION

In comparing the results of the tumor transplanting experiments, it is necessary to consider not only the contents of the solutions in which the tumor fragments were immersed before the inoculations, but also the lengths of time of the immersions. The temperatures were kept fairly constant, otherwise these also would have to be included. It will be seen that in discussing the contents of the solution, not only must the salt and its concentration be considered, but that the hydrogen ion concentration is of the greatest significance.

The favorable effect of immersion for twenty-four hours in solutions of pH 7.0 as compared with the more acid or more alkaline solutions is striking. Immersion in the pH 8.0 solution appeared to be less harmful than immersion in the pH 6.0 solution. Complete inhibition of growth was caused by the pH 6.0 and pH 9.0 solutions in twenty-four hours. From a histological examination of the tumor cells after immersion in solutions more acid or more alkaline than pH 7.0, it was found that there was a hydropic degeneration of the cells similar to that of tumor cells resulting from exposure to radium or to x-rays.

The action of the salts on the tumor tissue before inoculation is of interest. Sodium chloride had no effect in twenty-four hours, but exerted a harmful action in seventy-two hours. Lithium chloride produced a small inhibiting action in the twenty-four hour treatment. Calcium chloride caused a very strong inhibiting or retarding action on the subsequent growths even in ten hours. The use of potassium salts in the buffer mixtures showed that potassium exerted no specific retarding influence.

Cramer (4) several years ago studied the effects of sodium chloride and calcium chloride solutions on the growth of a transplantable mouse carcinoma. He allowed isosmotic solutions of these salts to act on the cells for one to two hours before inoculation. Marked inhibition of growth was shown by the cells immersed in the calcium chloride solution, practically none by those immersed in the sodium chloride solution. He found that the harmful effects of the calcium chloride could be overcome by subsequent immersion in sodium chloride solution, and that the action of the calcium chloride was only transient.

Although the experiments of Cramer are of interest in connection with the present work in showing a parallelism between the harmful action of the calcium salt and the less harmful action of the sodium salt, the following differences in the manipulations, aside from the use of mice in the one case and rats in the other, may be pointed out. In the present work, the hydrogen ion concentrations of the solutions in which the tumor fragments were immersed were controlled; in Cramer's work they do not appear to have been controlled. In the former, the inoculated animals were observed for six to seven weeks after inoculation; in the latter for two weeks. In the former, also, the times of immersion were varied for a number of the treatments, and certain salt mixtures were used. In spite of these differences, the results of Cramer offer valuable contributory evidence in connection with the transplantation phenomena.

The results of the transplantation experiments described in this paper are evidently due to cell destruction of some form. In view of the harmful action of the salts separately, and the harmlessness of the "balanced" mixture, the action on the cell membrane or wall appears to be the dominating phenomenon. Destruction of the membrane or cell wall, or perhaps better, modification of its permeability, destroys the reproductive power of the cell. The actions of small amounts of acids and of bases, and of various salts and mixtures of these, have been described in connection with other cell structures a number of times. In the present work, the effects of solutions of different hydrogen ion concentrations have been made more definite, and the relative action of sodium and calcium salts at a definite hydrogen ion concentration brought out clearly. The explanation of these actions is, however, the same as that developed in other connections by a number of workers.

The work on the protease actions of malignant human and rat tumor extracts presented elsewhere (1) shows some interesting similarities and differences as compared with the transplantation phenomena presented here. While it is true that the causes underlying the two sets of phenomena are probably of entirely different nature, the protease results will be outlined briefly in comparison with the transplantation results.

The optimum hydrogen ion concentration for protease action and the favorable medium in which tumor fragments on immersion retain their ability to grow when transplanted, correspond very closely to that represented by pH 7.0, unfavorable conditions for both being reached more rapidly on the acid side than on the alkaline side. Chlorides of the alkalies in fairly dilute solution and within short time limits, did not affect appreciably the protease action or the ability of tumors to grow when transplanted, but calcium salts under similar conditions exerted a very marked inhibiting or retarding action on both. On the other hand, a definite difference is observed with a mixture of sodium chloride, calcium chloride, and potassium chloride in the concentration found in Locke-Ringer's solution. Retardation of the protease action was caused to the same extent that the salts separately would cause such action, while with the transplantations, the salts exerted an antagonistic action toward each other in the sense that no inhibition of the tumor growth was observable in the subsequent inoculations.

The two sets of actions, therefore, can be ascribed to different causes; the transplantation results in the first instance to salt actions on the permeability of the cell membranes, the enzyme results to chemical actions of unknown nature on the enzyme molecule or enzyme grouping of some molecule. The comparative actions of the sodium and calcium salts parallel each other when used alone, but not when used in mixtures in certain proportions and concentrations. The parallelism, even if due to different causes, as is probable, is of interest.

Certain facts may be mentioned in this connection, even if they have no direct connection with the phenomena described in this paper. In reviewing past work on the inorganic constituents of neoplastic tissue, it may be stated that the calcium content of rapidly growing tumors was found to be small. Calculating the potassium-calcium ratio of such tumors, large concentrations of potassium as compared with the calcium were found, while in old, necrotic, or slowly growing tumors, the concentration of calcium was considerably greater in comparison with that of the potassium (5).

Finally, it may be stated that while a definite connection may exist between the conditions affecting the permeability of cell membranes, the factors which influence the activities of intracellular enzymes, and the relations of the inorganic constituents of neoplastic or other tissue, such a connection is still obscure and can only be hinted at until more definite experimental evidence is available.

4. SUMMARY

The growth of the Flexner-Jobling rat carcinoma was investigated after grafts had been immersed in solutions of various salts of different hydrogen ion concentration. Calcium strongly inhibits growth, and a pH of 6.0 appears to be more harmful than pH 8.0.

The growth of the tumors was compared with the protease action of extracts of malignant human and rat tumors, and similarities and differences in these phenomena indicated, as well as possible explanations for them.

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PLATE 1

The plates show three photomicrographs taken with the same magnification.

Plate 1 shows untreated tumor tissue of the Flexner-Jobling rat carcinoma.

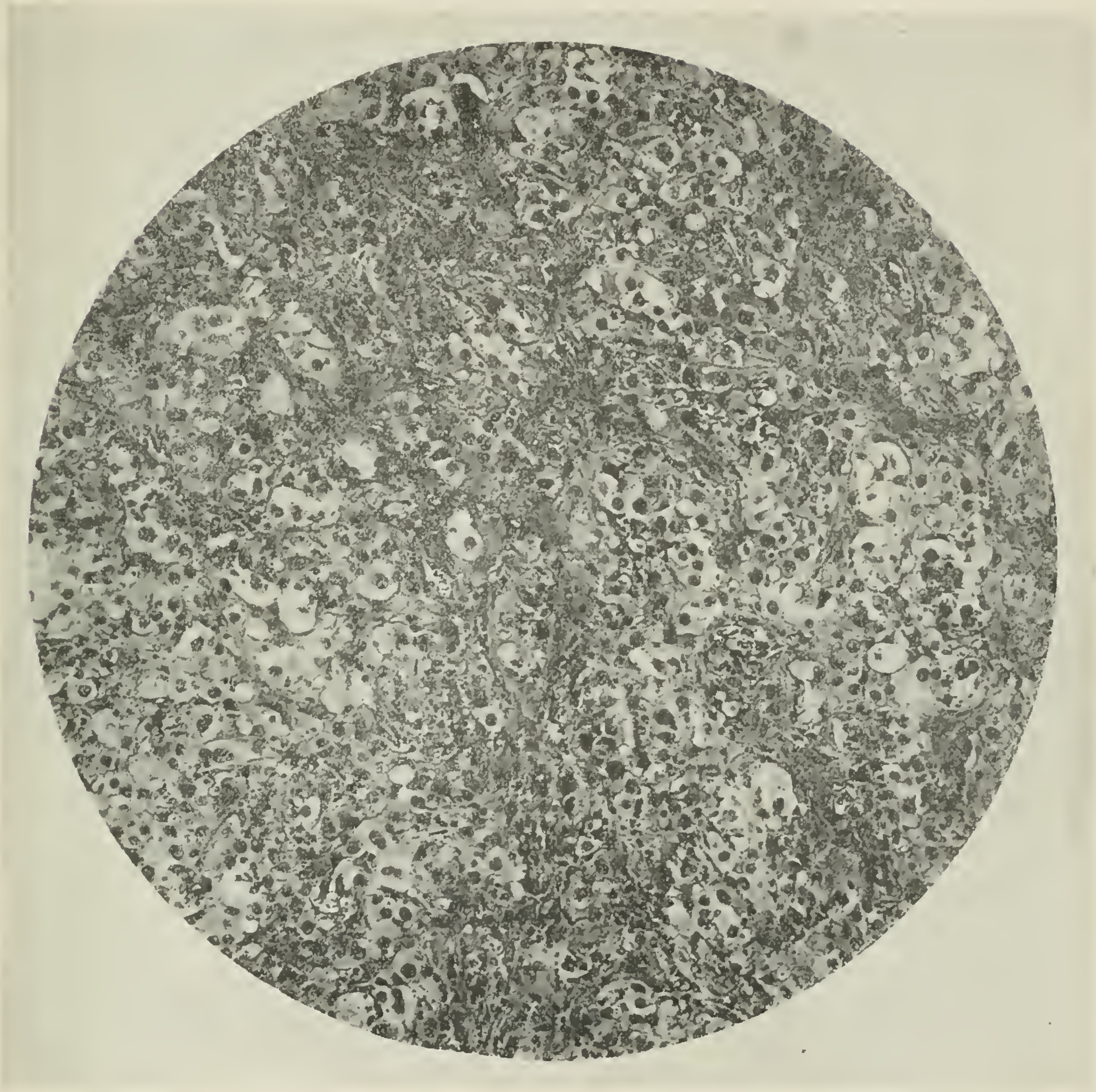


PLATE 2

Plate 2 represents the same tumor after immersion for twenty-four hours in the phosphate mixture, having the pH 5.8.

GROWTH OF FLEXNER-JOBLING RAT CARCINOMA

K. SUGIURA, H. M. NOYES AND K. G. FALK

PLATE 2

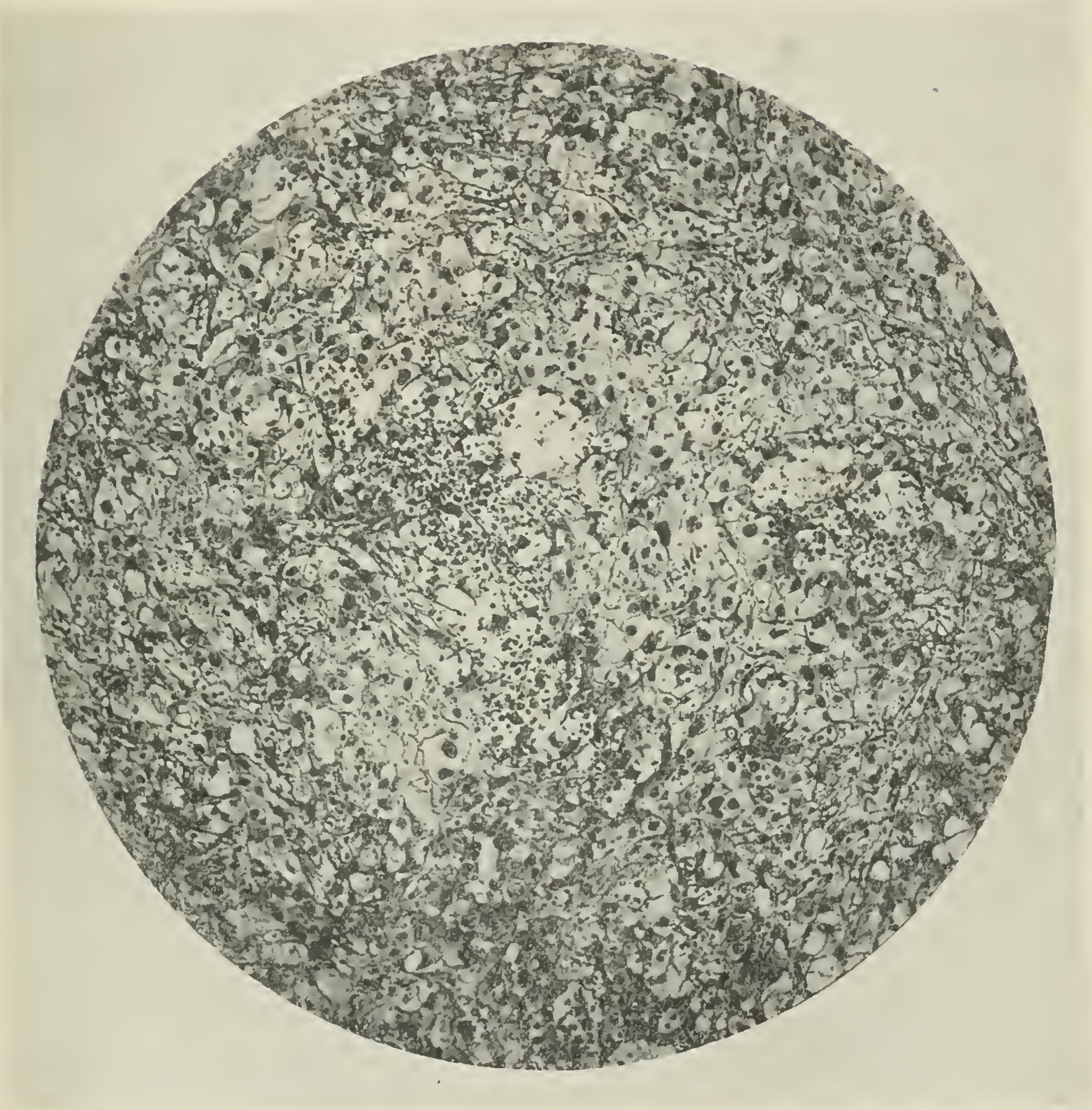
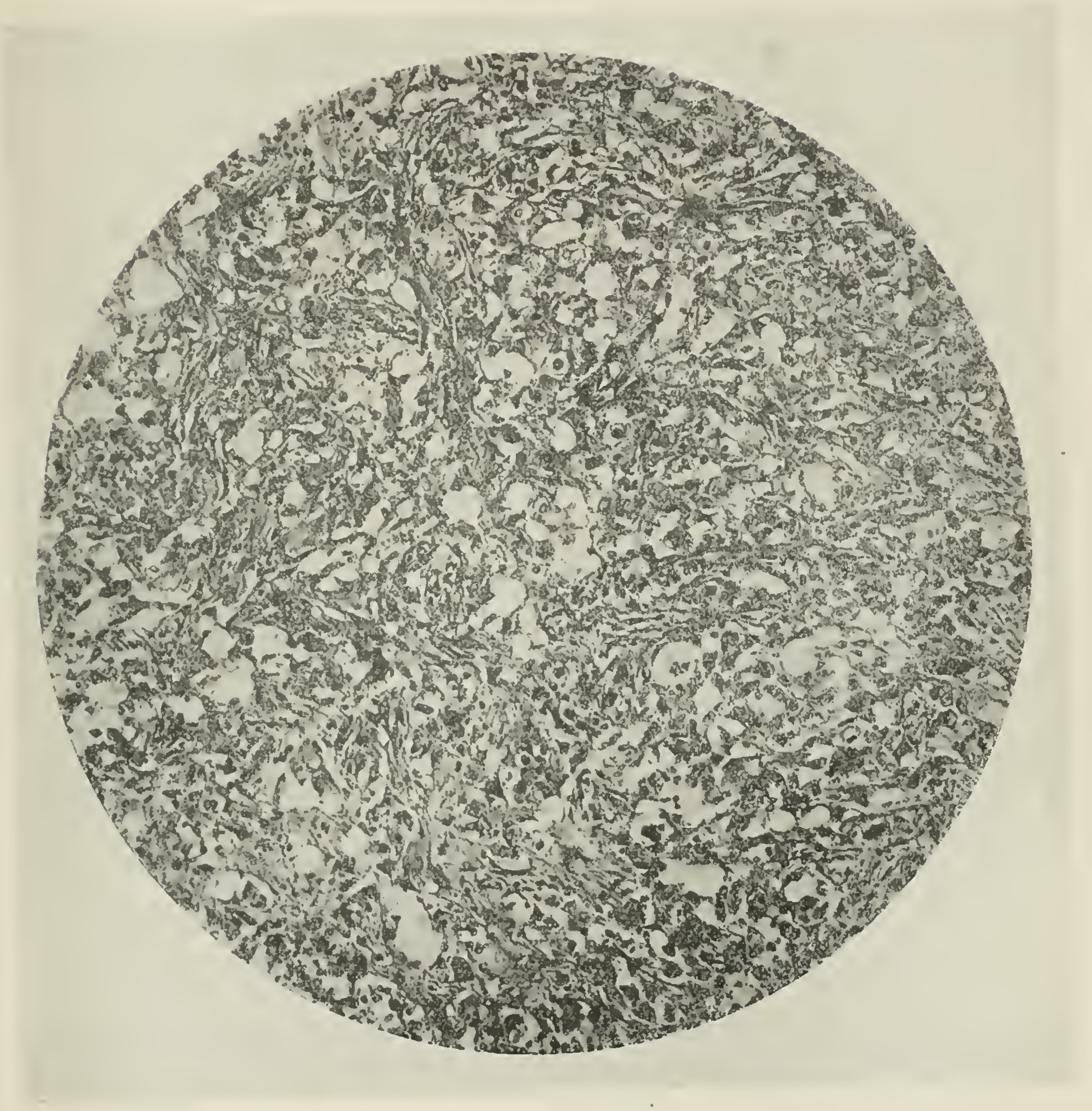


PLATE 3

Plate 3 shows the results after immersion of the same tumor for twenty-four hours in the 0.078 M calcium chloride solution, having the pH 7.0. Microscopical examination of the treated tissues showed considerable degenerative changes. (For further reference see text.)



PRIMARY SPONTANEOUS TUMORS IN THE KIDNEY AND ADRENAL OF MICE

STUDIES ON THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE

Seventeenth Communication

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From the Otho S. A. Sprague Memorial Institute and the Department of Pathology of the University of Chicago

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Primary tumors of the kidney occur not infrequently throughout the animal kingdom, and, in general, seem to exhibit the variations and peculiarities seen in human renal tumors. As an indication of the comparative pathology of renal tumors the following review of the literature is presented:

Mouse: Few cases of primary renal tumors have been described among the great numbers of other tumors found in this species. Tyzzer (1) found 4 renal growths, which he interprets as hypernephromas, among 83 primary tumors in mice. One of these mice, an old female, had also an adenoma of the lung, a carcinoma of the ovary, and a lymphoma infiltrating both kidneys; another had an adenoma in the lung. At the time this paper was written the interpretation of hypernephroma was more liberal than at present, and the illustrations might now be interpreted by many pathologists as papillary adenoma of the kidney, which was Tyzzer's original diagnosis in two of his cases.

Among the 300 mice with spontaneous tumors described by Haaland (2), there were but two renal tumors. One was a large growth in the kidney of a twenty-two months old male mouse, and had invaded a vein but produced no metastases; microscopically the structure was that of an adenocarcinoma. The second tumor, in a mouse of the same sex and age, resembled perfectly in structure a human hypernephroma; there were no metastases, although the lung contained an adenoma. Inoculation of the hypernephroma into 40 mice was without result.

Murray (3) also described a spindle-cell growth surrounding the kidney without infiltrating it, apparently a primary retroperitoneal sarcoma and related to the kidney by position only.

Further than the above we can find no reports of primary renal tumors in mice, although Stumpf (4) has contributed a discussion of the behavior of carcinomas inoculated into the kidney.

Rats: Of 103 tumors found in 100,000 rats autopsied in plague work by McCoy (5), 11 were in the kidney, and were classified as 4 adenomas, 6 carcinomas, and 1 papilloma. Woolley and Wherry (6) in 23,000 rats found 22 tumors, of which 3 were in the kidney; all were of renal-cell type. Bullock and Rohdenburg (7), in a compilation of the literature on rat tumors, found 123 (including those cited above) of which 7 were fibroadenomas, 1 a papilloma, and 8 carcinomas of the kidney. Among 32 cases of their own in laboratory white rats, there were 10 adenomas of the kidney. Therefore, of 155 rat tumors 26 were of renal origin. A further case of carcinoma of the kidney in a wild rat was reported by Beatti (8). Loewenstein (9) described tumor-like papillary outgrowths arising in the pelvis of the kidney, as well as in the ureter and bladder, of rats infected with Trichodes crassicauda specifica.

Since sarcomas are much more common than carcinomas in rats, the fact that all the renal tumors in rats are of epithelial structure is of interest.

Squirrels: In 250,000 ground squirrels (Citellus beecheyi) autopsied in plague work, McCoy (10) found 8 tumors, of which one was described as an angiosarcoma of the kidney. A case of hypernephroma in the kidney of a grey squirrel (Sciurus carolinensis pennsylvanicus) was reported by Fox (11).

Birds: Fowls are rarely subject to renal tumors. Of 880 examined by Curtis (12), 79 had tumors, 5 of which were in the kidneys; but these were not microscopically corroborated and there is reason to doubt their true neoplastic nature. In 34 cases of tumor in fowls compiled by Wernicke (13), not one was primary in the kidney although in 3 cases renal metastases were found. In 852 autopsied fowls Bürger (14) found 12 tumors of which none was in the kidney, although a sarcoma of the ovary had produced metastases in the kidney in one case.

The review of the literature on tumors in fowls and birds by Joest and Ernesti (15) who collected 112 cases and added about 50 more, reports no further renal tumors.

Other birds seem to have renal tumors perhaps more frequently, as is indicated by the following reports: White (16) described a fibrosarcoma in the kidney of a goose. Fox (17) reported in the kidney of a male chestnut eared finch (Amadina castanotis) a medullary carcinoma, becoming scirrhous in places, with metastases to the lungs, and three cases of benign adenoma of the kidneys in undulated grass parrakeets (Melopsittacus undulatus). He had previously reported (18) two cases of papillary cystadenoma and also a "cyst adeno-carcinoma papilliferum"(19) in the same species; besides an adenocarcinoma of the kidney in a saffron finch (Sycalis flaveola), (20) and a spindle-cell sarcoma of the left kidney with metastasis into the left tibia in a scaly ground dove (Scardapella squamosa) (21). Seligmann (22) reported as a diffuse carcinoma a growth which involved both kidneys, with metastases in the liver and mesentery, in a Chilian pintail (Dafila spinicauda) which was twenty-six years old. Baird (23) reported a case of keratinizing epithelioma in the kidney of a fowl, and Borrel and Masson (24) also described a renal tumor in a fowl which showed both cylindrical and squamous elements.

Domesticated mammals. Rabbits, which are less subject to tumor than most mammals, seem to have a relatively large proportion in the kidney, for Scott (25) stated that of 39 new growths reported, 5 were benign renal adenomas, resembling Wilms' tumors in structure.

Swine also seem to be particularly subject to renal tumors, for of the 12 cases of tumors in swine collected by Sticker, 7 arose in the kidney, and other cases reported since then indicate the same tendency. Many of these tumors were of the mixed embryonal tumor type.

In horses renal tumors are far from rare. In Sticker's (26) compilation, of 509 cases of malignant tumors in horses 37 were in the kidneys. McFadyean (27), in 63 cases of tumor in animals included 5 in the horse kidney, none with metastases. Of 142 equine neoplasms among 77,224 horses slaughtered in Japan, there were 9 in the kidney, as compared with 49 in the testicle (Kimura) (28).

According to Trotter's (29) figures, renal tumors are much less frequent in cattle, for of 305 cases but 1, a colloid cancer, arose in the kidney; but Sticker's figures show 10 of 78 bovine tumors in the kidneys. Steinke (30) described as hypernephroma a tumor of the kidney in a cow. Roussy and Wolf (31) in their review on cancer in animals, gave a picture of a tumor from a bovine kidney resembling a hypernephroma, and stated that cancer of the kidney in horses resembles that in man, while in swine the adenosarcoma of the kidney is among the most common tumors. Cadiot (32) quoted a case of an enormous cancer of the kidney in a mare.

No reports of cases of renal tumors in sheep can be found.

Of 766 primary cancers in dogs in Sticker's tables 19 were in the kidney. McFadyean also described two renal tumors in dogs, each case exhibiting metastasis. No renal tumors appeared in the 21 cats with tumors in Sticker's lists, nor in Roffo's 7 cases (33) nor in Murray's 11 cases (34) but Teutschlaender mentioned the cat (Kater) on his list of animals in which carcinoma of the kidney has been reported. Murray also described 48 cases of tumors in dogs, 12 in horses, and 18 in cows, with none arising in the kidneys.

We are indebted to Dr. L. E. Day for his summary of 316 tumors found among 2000 animal specimens sent to the Chicago Laboratory of the Bureau of Animal Industry. These do not represent all the tumors that are observed in the slaughter house, but merely specimens sent to the laboratory by the inspectors when in doubt concerning the diagnosis. Among 175 tumors of cattle there were no renal tumors although there were four adrenal tumors, two diagnosed as sarcomas and two as hypernephromas. Among 93 tumors in pigs no less than 52 were in the kidney, 47 being diagnosed as embryonal adenosarcomas and 5 as sarcomas; there were no adrenal tumors. In considering the tumors in swine it is to remembered that nearly all swine are slaughtered before they are two years old, so that not many of the slaughtered animals have reached an age for developing carcinomas. Among 48 tumors from sheep none whatever were found in the kidney or adrenal. These figures emphasize the infrequency of renal tumors in cattle and sheep and the frequency of mixed tumors of the kidney in swine, which resemble the typical mixed tumors of the human kidney.

Other incidental cases are: Sarcoma of the kidney in a swine (Hamburger) (35), the report of which leaves some uncertainty whether the growth may not have been leukemic.

Papillary adenoma in the left kidney in a mongoos lemur (White) (36). A tumor involving the kidneys, liver, lungs, and ovaries of a python, the primary site not being determined (Bland-Sutton) (37). A leiomyoma in the kidney of a horse, described by Wells (38). Pick (39) described a soft adenoma involving both kidneys of an eel. Williams (40) stated "A malignant renal tumor (derived from an adrenal 'rest') has been described by Bland-Sutton in a marmot." In Teutschlaender's compilation (44) were listed the following cases from the literature: Hemangioma of the kidney in a horse; carcinoma of the kidney in fowl, frog, pike, and buzzard.

While the above compilation is certainly not entirely complete, it serves to bring together much of the literature on the comparative pathology of renal tumors, and to indicate their general distribution and frequency.

Adrenal tumors. We can find no reports of primary adrenal tumors in mice, despite the great numbers of mice that have been examined at autopsy and the not infrequent occurrence of adrenal tumors in other species. Thus, Kimura (42) found in the literature records of adrenal tumors in 24 horses and 46 cattle, and he himself found 5 adrenal tumors among 46 tumors from horses. The extensive discussion of the comparative pathology of adrenal tumors by Steinke (43) mentioned tumors only in horses and cows, commenting on the absence of such tumors in other species except for an adrenal tumor of parasympathetic-cell character in a two-year-old swine, reported by Klawitter.

There is, however, the interesting case reported by Smallwood (44) as a carcinoma of the kidney of a frog and believed to be derived from the adrenal tissues. Murray, who reëxamined this specimen, corroborated its carcinomatous nature, but his statement suggests that he was not convinced that it developed from adrenal rather than renal cells. The illustrations in Smallwood's article suggest a papillary renal tumor.

Fox (45) described as a hypernephroma a tumor that arose in the adrenal of a California hair seal (Zalophus californianus), and also described a hypernephroma in the adrenal of a brown cebus (Cebus fatuellus) (46).

As stated previously, in the 316 animal tumors examined by Day in the Chicago Stock Yards, but 4 arose in the adrenal, all in cattle, 2 being diagnosed as sarcoma and 2 as adenoma.

RENAL TUMORS IN MICE IN THE SLYE STOCK

In 33,000 autopsies performed on mice of the Slye stock, but 16 cases of unquestionable primary tumor arising in the kidney have been observed, supporting the impression that the kidney of the mouse is not among the common sites of primary neoplasm. These renal tumors are, according to the histological evidence, to be classified as follows: 1 carcinoma, 3 adenomas, 1 hypernephroma, 7 sarcomas, 3 mesotheliomas, and 1 sarcoma in the renal pelvis. It will be noted that we have observed no instances of the mixed tumors of the kidney that are so common in man and some other species, nor have we had any papillary tumors of the renal pelvis. The features presented by these several types of renal tumor are best described by giving briefly the findings in each of the several cases that we have observed.

EPITHELIAL TUMORS OF THE KIDNEY

1. Primary carcinoma of the kidney (fig. 1). Female mouse (1934). This mouse showed a white solid mass in the right kidney about the size of a normal kidney. Beyond this there were no

abnormalities found, except that the spleen was about three times the normal size. The left kidney was normal, and no metastases were found. Microscopically the tumor is composed of cells of epithelial type, but without the characteristics of either adrenal cells or of tubular epithelium of the kidney. They are arranged in large, pseudo-alveoli with much central necrosis, so that the persistence of the tumor cells near the bands of stroma gives in places the impression of a papilloma. Although the growth does not infiltrate the kidney very much, it has no capsule of its own, infiltrates the renal capsule in places, and is undoubtedly

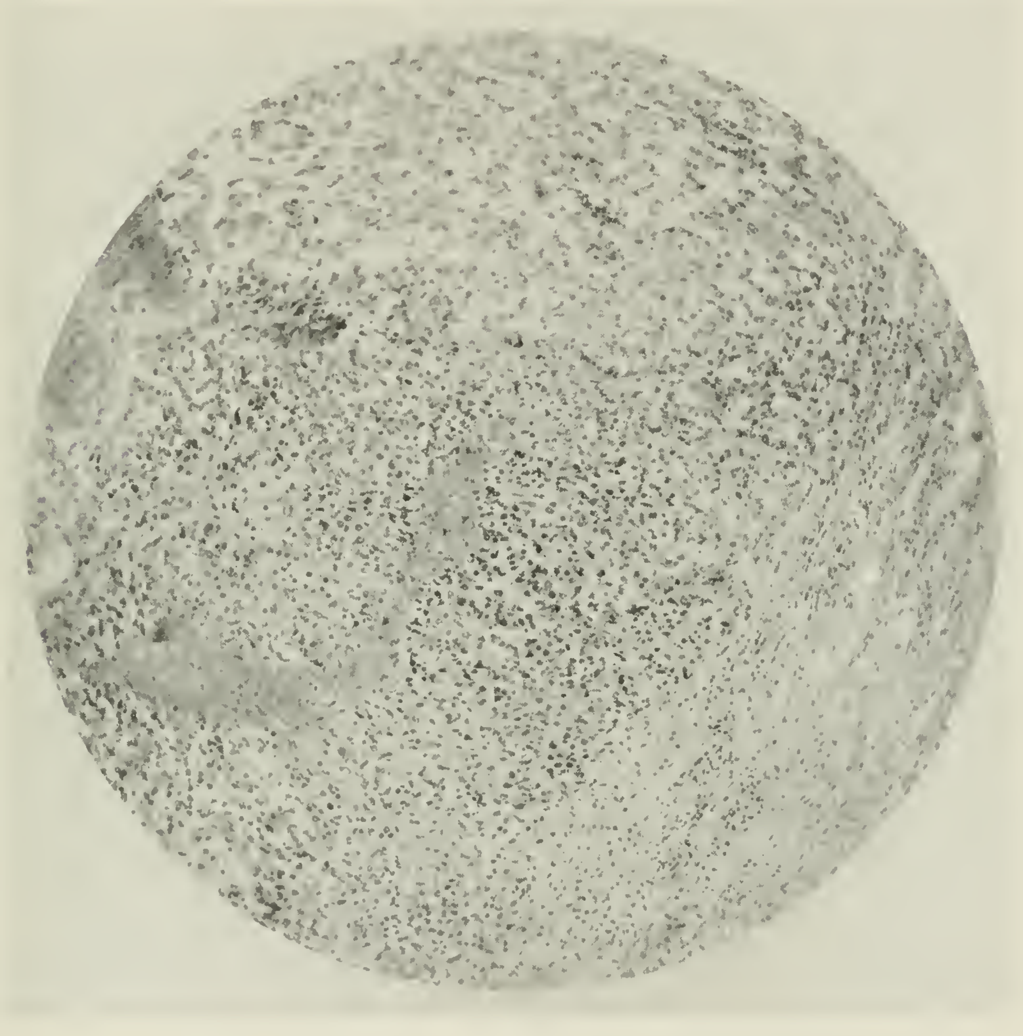

Fig. 1. Primary Carcinoma of the Kidney

The junction of the compressed renal tissue and the tumor is shown. Apparently this tumor is derived from renal epithelium. Mouse 1934. × 110.

malignant. Evidently it is a tumor derived from the renal epithelium and may be properly designated as a carcinoma, although the term mesothelioma might also be appropriately used. Presumably it is in an early stage of malignancy in view of the relatively small amount of extension of the growth.

2. Solid adenoma arising in bilateral cystic kidneys. Male mouse (9907), age two years, thirteen days. Both kidneys were converted into masses of small cysts of various sizes, resembling the congenital cystic kidneys, although no cysts were found in the liver. Some of the cysts contain colloid masses, and between them are occasional foci of small round cells. There remains more kidney tissue in a functional condition than is usually seen in fatal human cases of congenital cystic kidney, but this tissue is far from normal, the tubules containing many hyaline casts, the interstitial tissue being infiltrated with round cells, and many of the glomeruli being more or less hyalinized. In the lower pole of each kidney was a fleshy nodule about 3 to 4 mm. in diameter. Microscopically these nodules are composed of a solid tissue, made up of groups of large epithelial cells somewhat resembling renal epithelium. About these is a delicate stroma containing some collections of small round cells. The nodule is distinctly encapsulated and seems to be in the renal substance rather than within one of the cysts. In many respects the structure resembles that of the benign ovarian adenomas of mice (47). No abnormalities of importance were noted in the other organs.

3. Adenoma of the kidney (fig. 2). Male mouse (10220), which died with advanced sarcosporidiosis; had marked chronic nephritis, both kidneys being large and nodular, with atrophic areas alternating with areas of swollen and dilated tubules, some of the latter being distinctly cystic. Beneath the capsule of one kidney was a nodule 4 mm. in diameter, with a well defined capsule. It consists of a mass of large epithelial cells with solid cytoplasm, arranged in large cords with a very delicate stroma between them. The arrangement of the cells resembles that of the adrenal adenomas, and a diagnosis of benign hypernephroma might readily be made. The cells are less vacuolated than those

of the typical adrenal cortex, having a denser cytoplasm, and are not dissimilar to the epithelial cells found lining the distended renal tubules; it seems probable, therefore, that this adenoma is derived from renal epithelium. There are some clefts containing colloid material, and numerous calcific granules in the form of calcospherites.

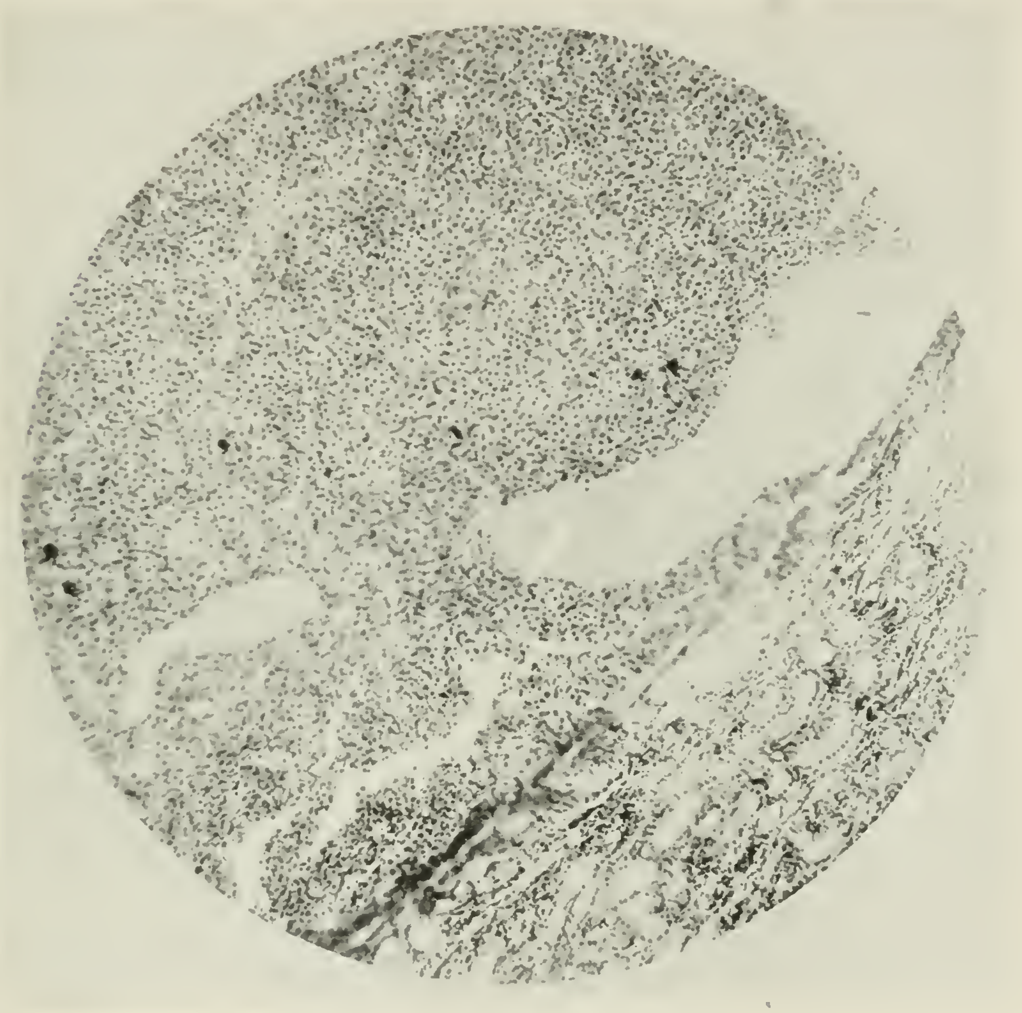

Fig. 2. Adenoma of Kidney

This somewhat resembles a tumor derived from adrenal cells, but is believed to have been derived from renal epithelium. Mouse 10220. × 65.

4. Adenoma of the kidney. Male mouse (24073), with a large liver cyst containing a tapeworm, exhibiting in one kidney a solid subcapsular nodule 5 mm. in diameter, with a delicate but definite capsule. This is composed of a solid mass of large

epithelial cells with abundant foamy cytoplasm and small dark nuclei. The cells are in large cords or alveoli with a very delicate stroma between them. There are numerous small foci of calcification of the tumor tissue. The foamy character of the tumor cells, together with their arrangement, suggests an adrenal origin for this growth, but the post-mortem changes have so altered the nuclear and cytoplasmic details that this cannot be determined positively. In places there is a tendency to tubular arrangement of the tumor cells, suggesting a renal origin. Outside the tumor the kidney shows an advanced chronic nephritis with many hyaline and fibroid glomeruli.

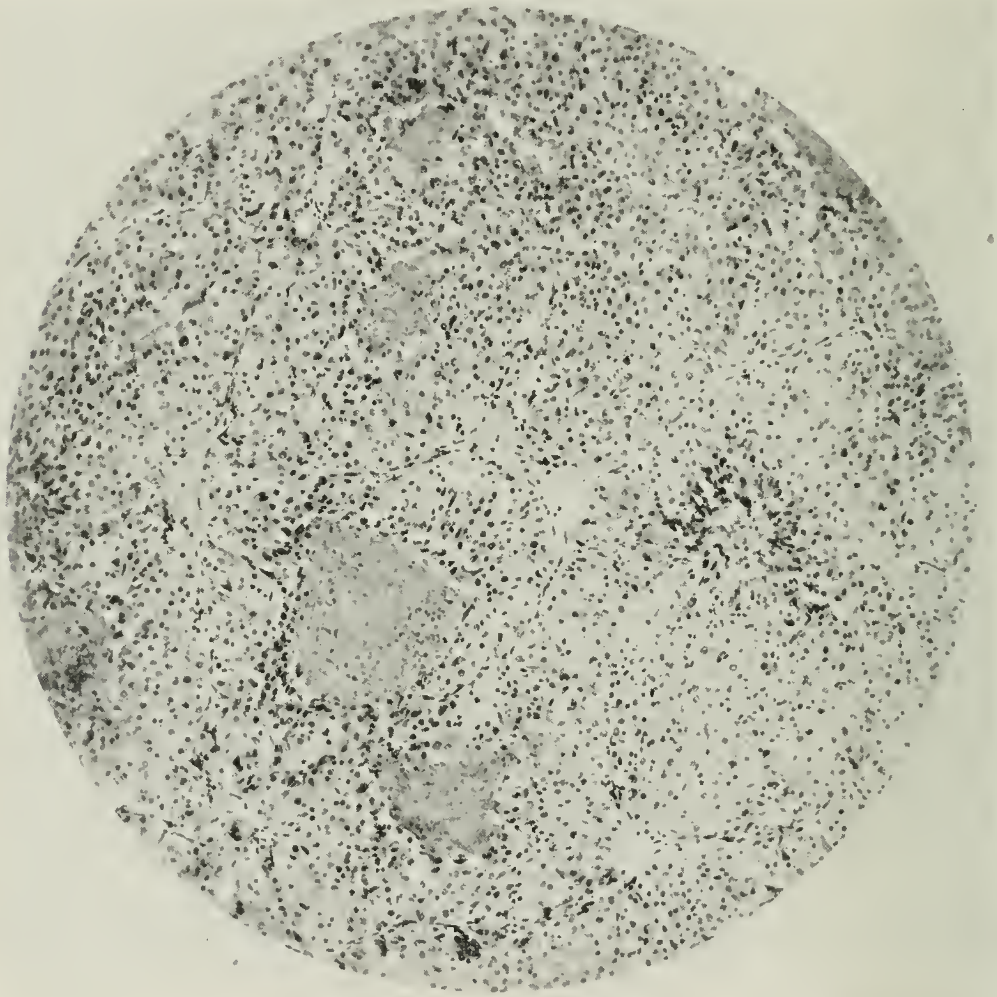

Fig. 3. Renal Hypernephroma

This growth entirely replaced one kidney, but produced no metastases; it corresponds in structure to human hypernephromas. Mouse 3639. × 110.

5. Hypernephroma in the kidney (fig. 3). Male mouse (3639) with no other lesions of significance. The left kidney was replaced by an encapsulated, hemorrhagic mass, measuring 22 x 22 x 18 mm. The right kidney was slightly enlarged and soft, and showed a moderate degree of chronic nephritis. No remains of renal tissue are found in the tumor mass, which presents the typical appearance of a hypernephroma. More than half the mass contains no cells, being composed of the residue of old hemorrhages and necrosis. The living portions consist of large foamy cells arranged in cords and alveoli with a delicate stroma. It is completely surrounded by a capsule which shows no invasion by tumor cells. In all respects this growth corresponds perfectly to the human renal hypernephromas. No metastases can be found.

In the hilum of the right kidney, attached to a large artery, is a nodule about 2 mm. in diameter which, in the center, resembles a small leiomyoma, but about it is a mass of granulation tissue. It bears no resemblance to the hypernephroma and its nature is unknown.

In view of the fact that chronic nephritis is one of the commonest diseases in mice, and appears in forms often quite similar to nephritis in man, it is strange that we have found so few instances of epithelial neoplasms in mice, especially the benign adenomas which are so often found in human kidneys showing chronic nephritis.

SARCOMA OF THE KIDNEY

The diagnosis of sarcoma, always difficult and often unsafe, is particularly dubious in the case of the kidney which presents so many non-sarcomatous growths that resemble sarcoma, and especially in the face of the statement made by Ewing (48): "Birch-Hirschfeld's group of adeno-angiosarcoma, derived from Wolffian remnants, and the lipomyosarcomas remain, however, the only well-defined varieties of renal sarcoma which have been fully divorced from a probable epithelial origin." Nevertheless, since the kidney contains connective tissue it is perfectly possible for sarcomas to arise therein, and in this mouse material we have several specimens for which only the diagnosis of sarcoma can be made, after excluding all other possibilities. In doing this we have carefully eliminated numerous growths of doubtful character, some of which also may really be sarcomas.[1] These cases are briefly described as follows:

6. Bilateral sarcoma of kidneys. Male mouse (7667), with both kidneys symmetrically enlarged to equal size, about 17 x 10 x 9 mm. They contained little recognizable kidney tissue, and were for the most part infiltrated by a fleshy, pinkish white tissue which involved equally the cortical and medullary portions. No lesions were found elsewhere. Microscopically both kidneys show infiltration replacing about 80 to 90 per cent of the renal elements. The neoplasm is composed everywhere of slightly oval cells, a little larger than lymphocytes, with deeply staining nuclei and very little cytoplasm. No evidence of neoplastic epithelial or mixed tumor elements can be found. This tissue infiltrates between the tubules much as do the cells in leukemic infiltrations, and the capsule is also invaded. The diffuseness of this infiltrative growth and its equal involvement of both kidneys makes it resemble a leukemia or pseudoleukemia, but this diagnosis is untenable in view of the lack of involvement of other organs or lymph-nodes. The diagnosis of sarcoma is made largely by exclusion.

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[1] We wish to quote here a statement of the criteria used in our consideration of sarcoma throughout this work as expressed in our paper on Primary Spontaneous Sarcoma in Mice (Jour. Cancer Res., 1917, ii, 1). "We recognize fully the difficulties that attend the differentiation of sarcoma, and for the purpose of this study have excluded every form of new growth concerning the nature of which there seemed any possible room for question. Therefore, we have not included numerous cases in which we think that the growths are probably sarcomatous, and many more in which we cannot be sure that the neoplasm is not sarcoma. On the other hand, the statistical value of our figures is lessened by the fact that we have undoubtedly omitted some growths that are true sarcomas. Our figures represent minimal values only. From the standpoint of investigations in heredity, with which our work is particularly concerned, it is just as undesirable to call a sarcoma something else as to include a granuloma among the sarcomas, and hence the rigid classification adopted in this study of sarcomas is no more satisfactory for our heredity statistics than would be a lax classification that included some growths of doubtful nature. Therefore, in charting the heredity statistics it is necessary to recognize the absence of positive criteria for the differentiation of sarcoma, and to admit the borderline cases with a mark of interrogation to indicate this fallibility."

7. Sarcoma of kidney. Old male mouse (24979), with the right kidney entirely replaced by a tumor, 16 x 12 x 10 mm., which infiltrated the adjacent tissues, including the ureter and the pelvis of the opposite kidney. The regional lymph-nodes were also invaded, and there was a mass in the root of the mesentery about 20 mm. in diameter, which seemed also to infiltrate the pancreas.

Microscopically, the right kidney is found to be almost completely replaced by a growth of large round cells, uniform in size, with very little cytoplasm, but with nuclei much larger than those of lymphocytes. There is no tendency to structural arrangement, the tumor cells infiltrating freely the tissues about the kidney, including the adjacent muscles and the pelvis of the opposite kidney, the ureter of which is surrounded by a mass of tumor. The regional lymph-nodes are replaced by tissue of the same character, but the mesenteric mass is completely necrotic, with few cells resembling those of the tumor. By virtue of its highly infiltrative character and the large size of the cells, this seems to be an undoubted sarcoma. The other tissues showed no evidence of either leukemia or pseudoleukemia.

8. Sarcoma of the kidney. Female mouse (26867) with much sarcosporidiosis, showed a marked enlargement of the left kidney, which was between two and three times the normal size, and infiltrated diffusely with a pink, fleshy tissue. Elsewhere in the body there were no important changes. Microscopically it is found that the enlarged kidney is infiltrated extensively, nearly all the renal elements being replaced by a tissue composed of round and elongated cells, considerably larger than lymphocytes and with more cytoplasm. They tend to be arranged in wide bands, growing out from the blood-vessels, but this arrangement is not constant or well developed. The neoplastic tissue grows out from the pelvis and tends to invade the adjacent tissues and the hilum of the opposite kidney. No similar tissue is to be found elsewhere in the mouse. The probable diagnosis is primary sarcoma of the kidney.

9. Sarcoma of the kidney. Female mouse (27148), with the right kidney much enlarged (18 x 15 x 32 mm.) and consisting of

a firm tissue overlaid with softer portions. Microscopically it is found that the kidney is diffusely infiltrated with a growth consisting of polyhedral cells, smaller than epithelial cells usually are, but with a little more cytoplasm than lymphoid cells. These cells exhibit no structural organization and have replaced all but a few of the original renal elements. About one-third of the tissue is necrotic. A small amount of the neoplastic tissue infiltrates the hilum of the left kidney, which also shows amyloidosis. No similar tissue is found elsewhere in the body, and there are no evidences of diffuse lymphoid hyperplasia or similar conditions. The lung contains a small benign adenoma.

The tumor shows much variation in the size of the nuclei, hyperchromatism being common, and occasional very large cells are seen. Unfortunately there is too much post-mortem change for exact details to be seen. The general character of the growth and the absence of similar changes in other tissues make a diagnosis of sarcoma seem inevitable.

10. Bilateral sarcoma of kidneys, with metastasis in spleen. Female mouse (396), with the uterus enormously distended with fluid because of vaginal obstruction by a seminal mass, and with great enlargement of both kidneys by a uniform white tissue. The right kidney measured 18 x 13 mm., the left 15 x 13 mm. On the anterior surface of the left kidney is a depressed scar. In the spleen there is a tumor nodule, 4 x 5 mm. No other tumor growths were found. The adrenals were normal.

Microscopically, both kidneys are found to be infiltrated diffusely, with replacement of 80 to 90 per cent of the renal tissue by a growth uniformly composed of small oval cells in solid masses, without any particular organization. No similar tissue can be found in any other organ except the spleen, which has a tumor nodule of the same structure as the renal tumor. The adrenals are in contact with, but not invaded by tumor. The tumor forms large masses in the hilum of one of the kidneys, or within the kidney itself. The left kidney is more involved than the right, and probably was the starting point of the sarcoma.

11. Lymphosarcoma of kidney. Female mouse (13124), with two separate mammary carcinomas, and in the left kidney a

nearly spherical mass about 14 mm. in diameter, which was partly necrotic. Microscopically this growth is composed of a dense mass of small round cells without visible cytoplasm, packed closely together without attempt at formation of any structure. It infiltrates the adjacent renal tissue freely, and there is a small amount of infiltration of the hilum of the opposite kidney. The structure is of distinctly neoplastic character, and there is no similar infiltration of other organs, or lymph-node enlargement, such as characterizes pseudoleukemia. The diagnosis of sarcoma, in structure corresponding to lymphosarcoma, is the only one that can be made on the features presented.

12. Lymphosarcoma of kidney. This seems to be similar to case 11, but unfortunately post-mortem decomposition has advanced so far that accurate microscopic study is not possible. Such tissue as remains stainable resembles a lymphosarcoma. The mouse was a female (12533) with no other tumors, and the right kidney was replaced by a soft pink mass measuring 14 x 12 x 12 mm. A small amount of the same tissue invaded the hilum of the left kidney.

MESOTHELIOMA OF THE KIDNEY

In our previous papers on tumors in the ovary (49) and testicle (50) of mice we have called attention to the not infrequent occurrence of tumors composed of polyhedral cells, presenting some characteristics resembling carcinoma and some resembling sarcoma, (figs. 4, 6, 7 and 8) and hence suitably designated mesothelioma, in accordance with Adami. These tumors are characteristic of the urogenital anlage, and hence it is not surprising that growths of the same structure are found in the adrenal and kidney. We have observed the following cases of renal tumors that seem to belong to this group.

13. This is a remarkable case in that a female mouse (21663), when *but one month old* exhibited two independent carcinomas of the mammary gland, and osteosarcomatous growths in the spinal column near the pelvis and in the left fifth rib. It lived but eighteen days more and at autopsy showed its left kidney also almost completely replaced by a tumor measuring 18 x 12 x

10 mm.; the right kidney, similarly involved, measured 10 x 6 x 6 mm. (fig. 4). These renal growths are entirely different in structure from the other four tumors. They are alike and vary in appearance in different parts, some portions resembling spindle-cell sarcoma while others are composed of larger, more polyhedral cells, arranged in a somewhat alveolar fashion, often separated by highly vascular septa. This structure corresponds to the type of growth often seen in tumors of the ovaries, testicles,

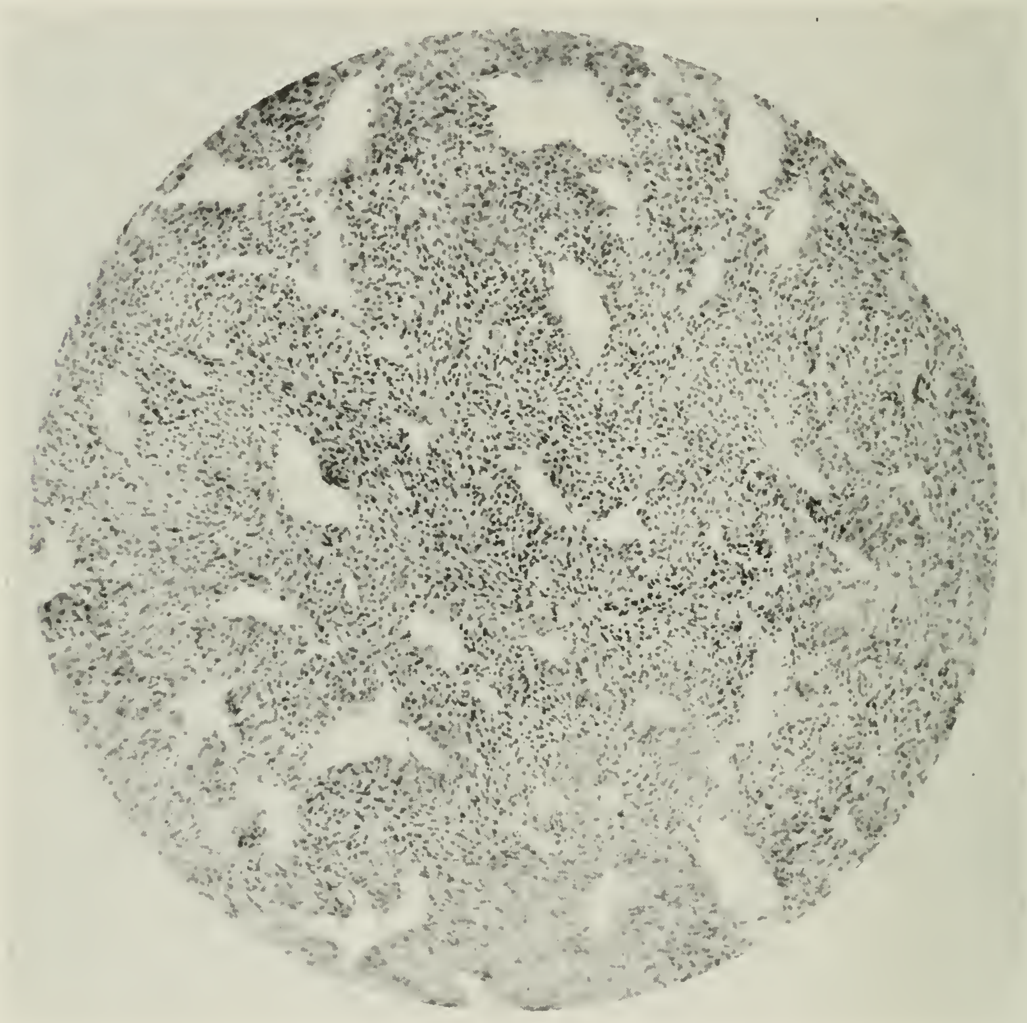

Fig. 4. Mesothelioma of Kidney

This portion of the growth presents a sarcomatous character; other portions exhibit more resemblance to epithelial growth. The tumor was found in a mouse but one month old, with four other tumors, which if not congenital must have developed very soon after birth. Mouse 21663. × 110.

and adrenals in mice, and agrees with the tumors called mesothelioma by Adami and Woolley. Since, except for this case, there have been very few other cases of malignant tumors arising in mice less than six months of age, the occurrence in so young an animal of at least four independent primary growths representing three distinct types of malignant neoplasm, is a most remarable condition, without, as far as we know, a parallel in either mouse cancers or in those of any other animal.

14. Male mouse (10011), with no other lesions of importance, had its right kidney largely replaced by a whitish tumor, forming a mass measuring 14 x 12 x 10 mm. Microscopically the tumor has largely replaced the kidney and has infiltrated the capsule in places. It is composed of small cells with a dark, round nucleus and a small amount of cytoplasm. These cells tend to form bands or pseudo-alveoli in some places, but for the most part the growth is composed of cells in a structureless mass. The left kidney was not involved by the tumor.

15. This seems to be similar to case 14. Male mouse (9779) had an enlarged left kidney without other lesions of note. Microscopically the kidney is largely replaced by a mass composed of small cells slightly larger than lymphocytes and with more cytoplasm, showing no structural arrangement. The main neoplastic mass lies at one side of the kidney, which it infiltrates slightly. The kidney itself shows some foci of round-cell infiltration. Some of these areas slightly resemble the tumor, but probably are not a part of it. There are some small areas of calcification, some scars, and numerous hyaline casts. Postmortem changes are too advanced for more accurate study. The opposite kidney contains no tumor, but there is the same amount of amyloid and calcification.

SARCOMA OF RENAL PELVIS

We have excluded numerous cases in which a retroperitoneal tumor of sarcomatous character has invaded the kidney hilum, but there is one case in which both the gross and microscopical findings distinctly indicate that the tumor had its origin in the tissues of the pelvis itself.

Female mouse (348) had a pale swelling extending downward and inward from the pelvis, composed of tissue of about the same consistency as the kidney and of uniform yellow color. On cross section the pelvis of the kidney was in the center of the mass formed by the tumor and the kidney, which measures 12 x 9 mm. The left kidney was of normal size with a pale area of swelling on the anterior surface. No other changes of importance were found except severe edema of the lungs.

Microscopically the growth is found to lie symmetrically about the pelvis of the kidney and the upper end of the ureter, invading the wall of these structures extensively. From here it passes along the vessels deeply into the kidney, but does not extend far into the cortex. The kidney is about one half as large as the tumor, the tubules being much dilated and the glomeruli more or less hyalinized. The tumor also extends some distance along the renal capsule as a thin layer. As no growths are found elsewhere it is evident that this growth arose in the tissues about the hilum of the right kidney. There is only a small amount of invasion of the left kidney hilum. The growth is composed of large round cells with a delicate reticulum. There are several delicate vessels packed with lymphoid cells, but no other evidences of lymphatic origin in this tumor. The cells of the tumor are polymorphous with considerable cytoplasm, and deeply staining nuclei much larger than those of the ordinary lymph-cell, and usually larger than the nuclei of the renal epithelium. No mitoses are seen. No changes of importance are found in the other organs.

This tumor is much less complex in structure than the cases of sarcoma of the renal pelvis in children described by de Vecchi and Salomon (51).

ADRENAL TUMORS

As mentioned above, no cases of adrenal tumors have hitherto been described in mice. We are able to report a few unquestionable instances of such tumors. Despite the frequency of benign cortical adenomas of the adrenal in man, we have found but one such tumor in mice. It may be recalled that adrenal tumors have not been found frequently in other species of animals.

Cortical adenoma of misplaced inter-renal adrenal rest (fig. 5). Between the kidneys of a female mouse (1921), with marked amyloidosis and chronic nephritis, was found a spherical mass about 5 mm. in diameter, attached neither to the kidneys nor to the intestines. Both adrenals were present at their normal site and were of normal structure and size, except for some peripheral round-cell infiltration.

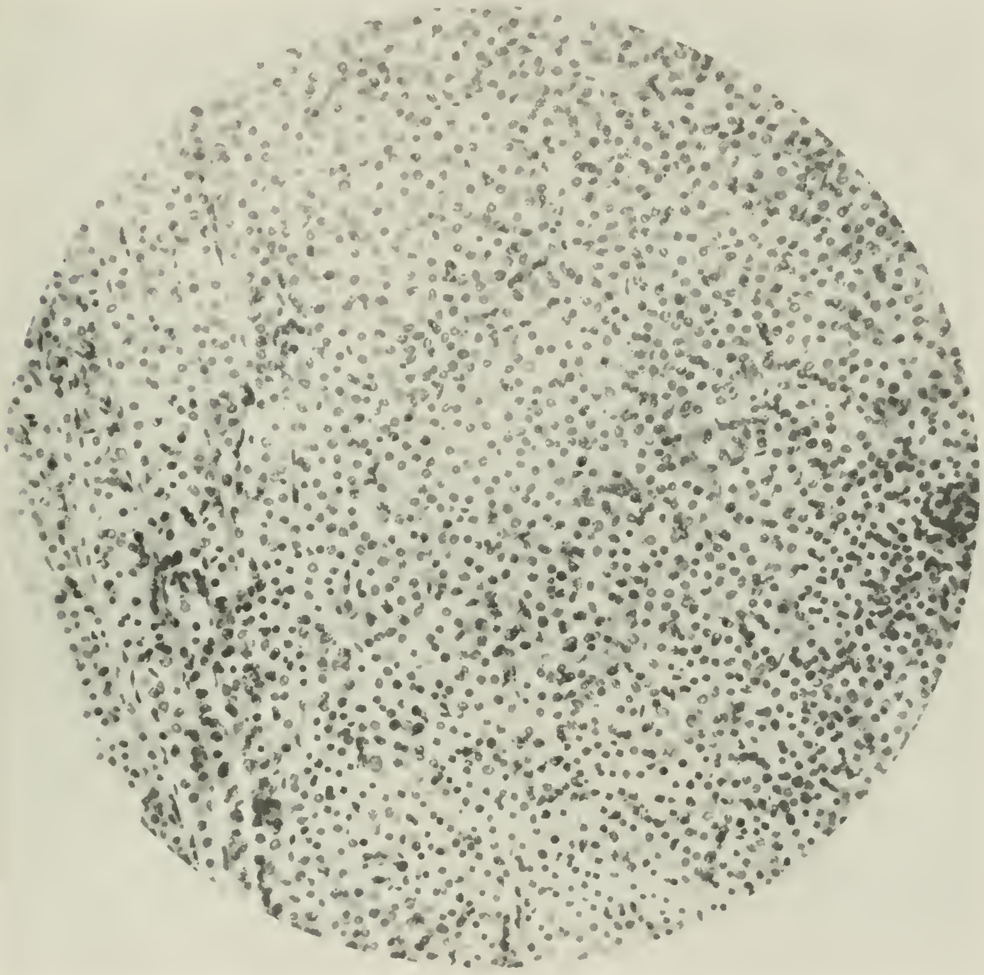

FIG. 5. CORTICAL ADRENAL ADENOMA

The tumor developed in an inter-renal rest of adrenal cortex tissue. A small zone of compressed adrenal tissue is seen at one side. Mouse 1921. ×225.

The tumor nodule is composed of a solid mass of cells resembling those of the adrenal cortex except in lack of orderly arrangement, closely packed together, and flattening out a thin shell of adrenal cortex, evidently all that remains of an adrenal rest, since no medullary elements are to be found. In all respects this tumor corresponds to the simple adenoma of the adrenal cortex seen in man.

MESOTHELIOMA OF THE ADRENAL

This seems to be the commonest tumor of the adrenal, as also of the testicle, of mice, and it is quite impossible to distinguish on the basis of microscopic appearance between the mesotheliomas arising in the different organs derived from the urogenital anlage. The cases in which the diagnosis seems certain are the following:

1. Male mouse (10390) had in place of the right adrenal a spherical mass 5 mm. in diameter, which is completely encapsulated and does not involve the kidney. No other nodules or findings of importance elsewhere. Microscopically the nodule contains no remains of adrenal tissue, but consists of a solid tumor made up of masses of large cells with considerable cytoplasm and large oval or spherical nuclei, arranged in atypical alveoli or broad bands with a very small amount of stroma containing thin-walled blood-vessels. In numerous places the capsule is infiltrated with tumor cells and there is some invasion of the areolar tissues about the adrenal, but no invasion of the adjacent kidney. This seems to be a typical mesothelioma of the adrenal in an early stage of malignancy.

2. Mesothelioma of adrenal with peritoneal metastasis. Female mouse (12744), with abdomen greatly distended by a bloody exudate, presented at the site of the left adrenal a whitish mass about the size of the kidney. All through the abdominal cavity are masses of partially necrotic whitish tissue, especially attached to the liver and uterus, which do not seem to be involved by this growth. There are enlarged retroperitoneal lymph-nodes, but elsewhere no lymphatic involvement. There are no tumors in the lungs.

Microscopically the tumor shows the usual features of the typical mesotheliomas, as described in the other cases, with slight tendency to alveolar arrangement. It does not seem to have infiltrated or produced metastases in the viscera. The right adrenal is surrounded, but not invaded, by the tumor and seems normal. The retroperitoneal nodes are replaced entirely by tumor tissue.

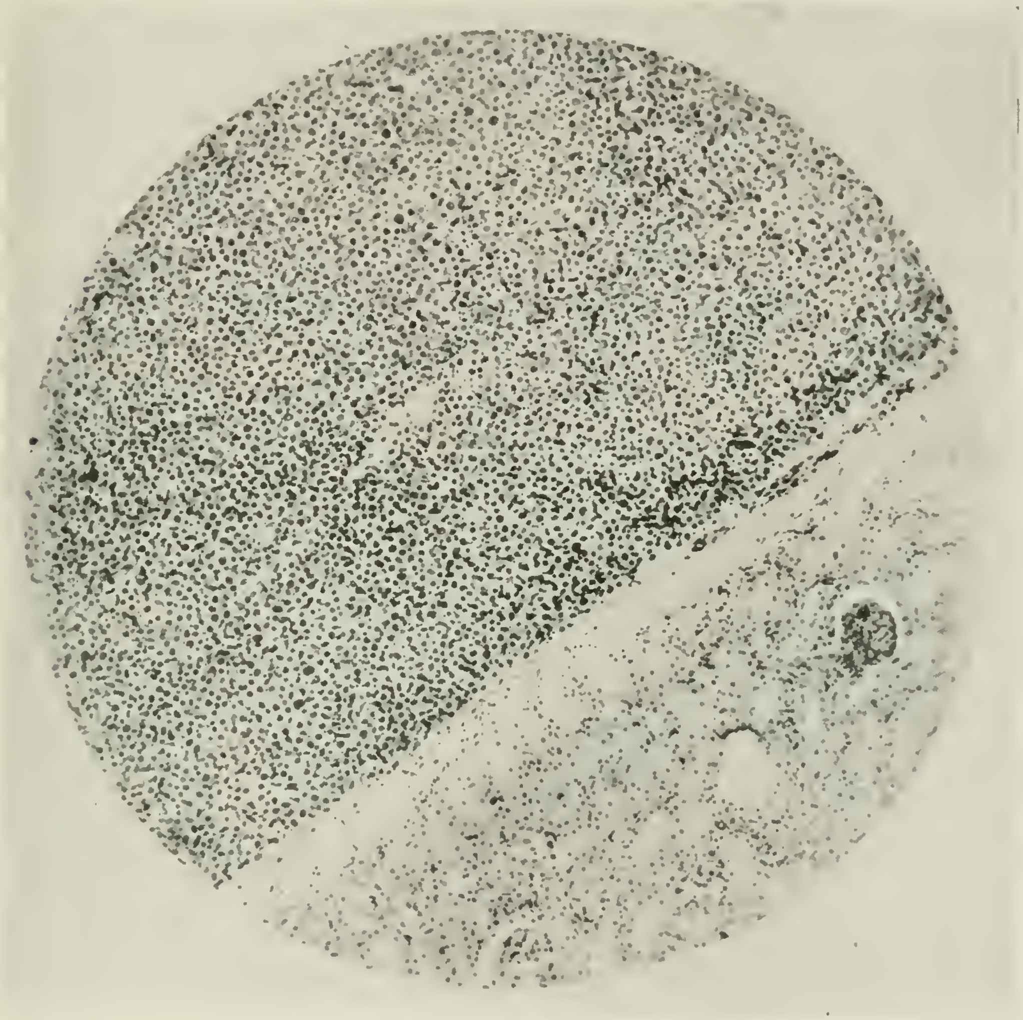

Fig. 6. Mesothelioma of Adrenal

This growth surrounded the lymph nodes and was widely disseminated through the perirenal tissues. Mouse 7699. × 110.

3. Bilateral malignant mesothelioma of adrenals (fig. 6). Female mouse (7699) with much subcutaneous edema, showed numerous enlarged subcutaneous lymph-nodes, some red and some pale, the largest measuring as much as 8 x 10 mm. There was a milky fluid in the peritoneal cavity, presumably because of pressure of enlarged lymph-nodes on the thoracic duct, for

the retroperitoneal nodes were greatly enlarged, up to 6 x 12 mm. The mesenteric nodes were also enormously enlarged, one mass at the root of the mesentery measuring 40 x 20 x 20 mm. Both adrenals were greatly enlarged, each being about the size of the kidney, which was adherent to the adrenal but not enlarged or infiltrated by neoplastic tissue. The uterus seemed to be infiltrated by tumor. The liver was not affected, and the lungs showed only a single small nodule, although there was some tissue increase in the upper mediastinum; both lungs showed a bloody edema, and there was a bilateral hydrothorax.

Although the gross appearance suggested a general lymphosarcomatosis or pseudoleukemia, yet the microscopic structure is of an entirely different character. Everywhere the neoplastic tissue presents the same appearance, being composed of a solid growth of cells with considerable cytoplasm, so that they look much like epithelium. The nuclei, which vary greatly in size, are much more solid than those of epithelial cells usually are. Mitotic figures are abundant. The invaded lymph-nodes are largely replaced by tumor cells, which are also found in the lymph-vessels of the lungs, but not in the liver. Both adrenals seem to be entirely replaced by the tumor, which lies upon the capsule of the adjacent kidney without any invasion of this organ. There are some areas of hemorrhage, but not much necrosis. The cells show no attempt at definite arrangement, but simply form a solid mass with numerous, poorly defined blood spaces.

This tumor resembles in structure other growths found arising in the urogenital anlage, and in view of this and of the complete replacement of both adrenals it is most probable that it did arise in these tissues, although the extensive lymph-node involvement is unusual in adrenal tumors. The structure is not essentially dissimilar to that of the adrenal tumor 10390, except for the amount of extension.

UROGENITAL MESOTHELIOMAS OF UNCERTAIN ORIGIN

Because of the identity in appearance of mesotheliomas from all organs originating in the urogenital anlage, it is not always possible to decide the place of origin of some tumors which involve two or more of these organs, as shown by the following cases.

12307. Mesothelioma of either adrenal or ovary. It is not possible to determine the origin of this tumor, which we have described in our paper on tumors of the ovary in the following words:

> The abdominal cavity shows several nodules whose exact origin is difficult to determine as the mate has partly devoured the body. The right ovary is, however, easily distinguished. It measures 18 x 12 x 12 mm. What seems to be the left ovary is 10 x 8 x 6 mm. There are 8 other similar nodules in the abdominal cavity, one being in the position of the left adrenal, measuring 10 x 8 x 8 mm. The other nodules are apparently in the mesentery. One lobe of the liver is converted into a tumor nodule 14 x 10 x 18 mm., irregular and lumpy in outline, pink in color.
>
> The tumor shows everywhere the same structure, consisting of irregular alveoli composed of large cells with abundant cytoplasm with well defined borders and deeply staining nuclei. Mitotic figures are numerous. The character is that usual to mesothelial growths. The ovary cannot be positively identified, but one mass exhibited in the capsule structure suggests compressed ovarian tissue with degenerated ova. In all respects this tumor is identical with the malignant ovarian tumors just described.
>
> It seems probable that this tumor arose in the ovary which exhibited the largest growth, but it is not possible to exclude the adrenal as the primary site.

The malignant tumors of the adrenal, ovary, and testicle commonly exhibit identically the same histological picture as that seen in this case.

We have also observed two other cases, described in the paper on ovarian tumors in which we cannot state whether the renal growth was primary or secondary.

12876. The left kidney contained a mass of pink, fleshy tissue, 18 x 14 x 14 mm. The right kidney, which was slightly enlarged, contained no tumor. The right ovary consisted of a pinkish tissue resembling that in the kidney, and measured

12 x 8 x 8 mm. In the mesentery was a similar, slightly paler mass, 16 x 8 x 8 mm. The retroperitoneal and subcutaneous nodes were not enlarged and no nodules were found in the lungs.

Microscopically the tumor is alike in all three places, consisting of a diffuse infiltrating growth of large round cells, which also invade the connective tissues about the kidney and ovary. It does not at all resemble the typical ovarian tumors, being apparently a round-cell sarcoma. We have no way of telling which of the three tumors was primary. The next case presents similar difficulties.

26. This mouse had a tumor mass about 8 x 10 mm. in the upper portion of the liver, with other smaller nodules near it. A similar small nodule was found in the right kidney. The right ovary was enlarged to two-thirds the size of a kidney, and was solid. Microscopically all these growths are composed of round cells, apparently a round-cell sarcoma. It is impossible to say which growth was primary.

Among several tumors arising in the retroperitoneal tissues, mostly of sarcomatous type, are a few of a structure identical with the characteristic mesothelioma type of growth that arises in the urogenital anlage. In the two cases described below this character of growth was so marked that it seems probable that the tumors have arisen in some misplaced embryonic rest, since the organs of this series were distinctly not the starting point of the growths. Numerous tumors of this sort have been described in man.

(22380) Malignant retroperitoneal mesothelioma with extension through the body wall. Male mouse presenting externally a subcutaneous tumor, involving the left hip and extending to the anus, the external measurements being 30 x 25 x 25 mm. When the body was opened it was found that this mass was an extension of a huge mass (40 x 20 x 18 mm.) which had surrounded the left kidney and pushed it to the ventral midline, invaded the spleen and pushed it mostly to the right of the midline by formation of a mass that measured 20 x 15 x 13 mm., and extended downward through the pelvic cavity where it surrounded the rectum and seminal vesicles and broke out through the body wall. There was slight infiltration of the lower pole of the right kidney. The liver was slightly enlarged and mottled with areas of infiltration. There was some tumor in the lower part of the mediastinum, and the lungs show many small spots of neoplastic tissue. There seemed to be no involvement of the lymph-nodes, either subcutaneous or abdominal. The testicles were not involved.

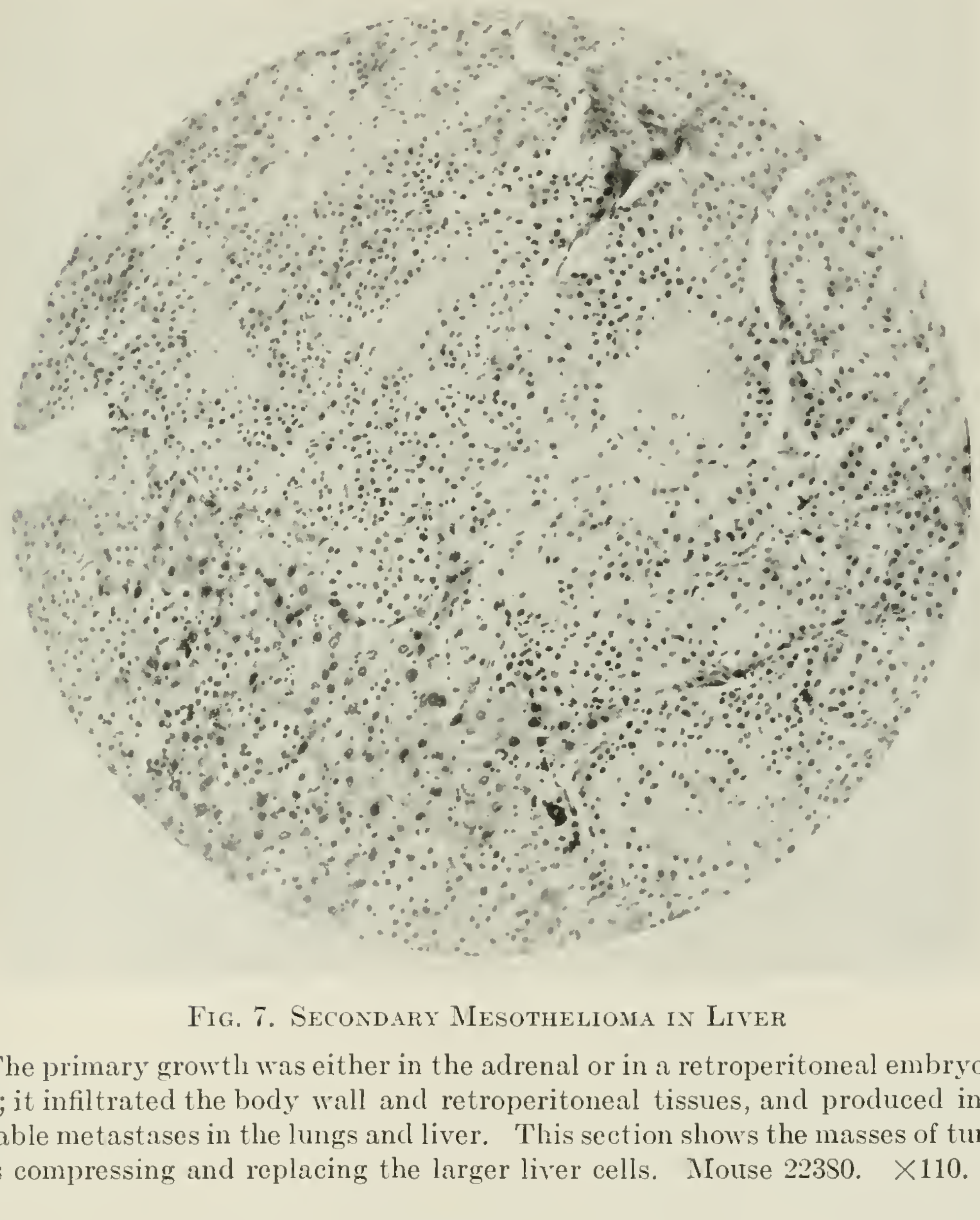

FIG. 7. SECONDARY MESOTHELIOMA IN LIVER

The primary growth was either in the adrenal or in a retroperitoneal embryonic rest; it infiltrated the body wall and retroperitoneal tissues, and produced innumerable metastases in the lungs and liver. This section shows the masses of tumor cells compressing and replacing the larger liver cells. Mouse 22380. ×110.

Microscopically the tumor everywhere consists of masses of large cells with much deeply staining cytoplasm, somewhat resembling liver cells, with a slight tendency to arrangement in cords or bands. Where the tumor invades the liver it can be seen that the cells are a little smaller and paler than the liver cells (fig. 7).

The growth infiltrates the liver widely, invades the muscle of the body wall, invades the renal capsule but not the kidney, infiltrates the abdominal sympathetic ganglia, surrounds densely the rectum and spreads into the seminal vesicle, invades the spleen, and in the lung appears as multiple tumor cell emboli within the vessels and as large tumor nodules largely replacing portions of the lung (fig. 8). The left adrenal was found entirely embedded in but not infiltrated by the tumor.

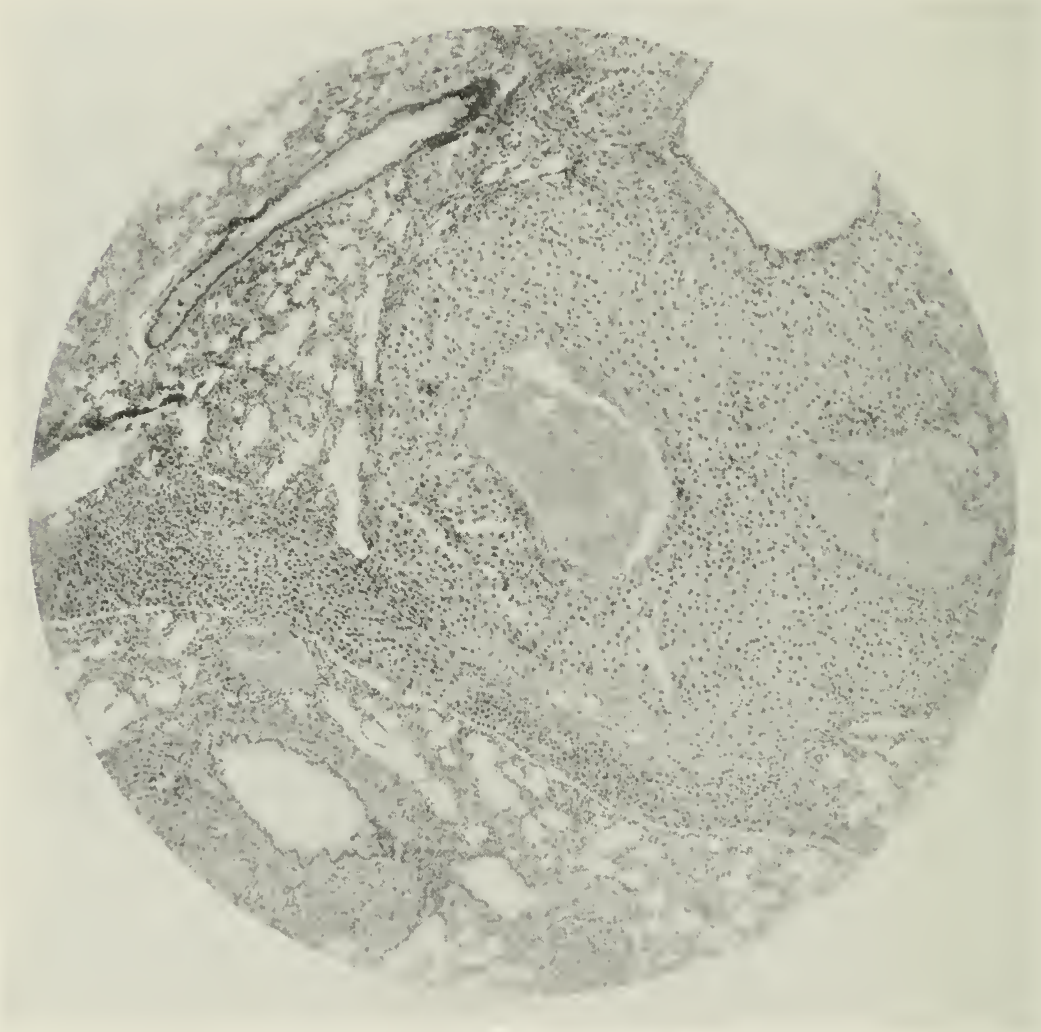

Fig. 8. Secondary Mesothelioma of Lungs

From the same case as figure 7. The section shows the extent of involvement of the lung. Mouse 22380. × 60.

The microscopic appearances are identical with those usual in malignant adrenal tumors, but the adrenal was not involved; and in view of the retroperitoneal origin, the growth may be presumed to have arisen in an embryonal rest of the urogenital anlage.

(9979) Malignant retroperitoneal mesothelioma infiltrating kidneys. Small female mouse with extensive infiltration of the right thigh by a typical spindle-cell sarcoma, and with a mass above the left kidney, about as large as the kidney itself, which was infiltrated by the growth. The entire mass measured 20 x 14 x 10 mm. The right kidney was not so much enlarged. The growth seemed to have arisen at the site of the left adrenal, and to have pushed the kidney forward. The liver was invaded by tumor and enlarged. No metastases were found elsewhere. Microscopically the tumor about the kidney bears no resemblance to the spindle-cell sarcoma of the thigh, being composed of a mass of polyhedral cells arranged in solid masses, with a slight tendency to form bands or cords. The nuclei are not much larger than those of lymphocytes, but the cells have much more cytoplasm. The chief mass lies above and behind the left kidney, which shows considerable invasion through the capsule and about the blood vessels. There is a similar invasion of the right kidney but much less tumor about it. The right adrenal cannot be located; the left is free from tumor although there is a necrotic area between it and the kidney. The liver shows a large nodule of the same sort of tumor.

SECONDARY TUMORS OF THE KIDNEYS AND ADRENALS

The mouse kidneys seem to be extremely insusceptible to metastatic invasion by tumors. In our entire series of primary tumors of mice, now in the neighborhood of 5000 cases, of which the predominating form is carcinoma of the mammary gland, we have never met with a metastatic growth from one of these tumors into the kidneys, with one possible exception, despite the frequency of pulmonary metastates, which often replace most of the lung. The only metastatic carcinomas of the kidney that we have seen have been found in four cases of primary carcinoma in the lung (3098, 11777, 12373, 14242). A photograph of the first of these four cases appears in our paper on lung tumor (52). These carcinomas of the lung are especially likely to produce extensive metastases, and the suggestion of some authors that these pulmonary growths in mice should not be included among the true tumors is evidently based on ignorance of their character. The fact that they have furnished our only instances of metastatic carcinoma in the kidney is sufficient evidence of their true neoplastic character and the high malignancy of some types.

Although we have seen many instances of widespread metastasis in sarcoma, the kidneys are almost immune from vascular sarcoma embolism. In our series of 87 cases of sarcoma in mice, although 23 showed metastasis but one hematogenous secondary nodule was found in the kidney, this coming from a mediastinal sarcoma (11791). Since then we have seen one other case of vascular metastasis of sarcoma into the kidney, from a sarcoma of the uterus (12058), with a large nodule of the same structure almost replacing the lower pole of the right kidney, and metastasis in the right ovary. Even the widespread growths of small round cells, which resemble the condition called lymphosarcomatosis in man, seem to affect the kidney but little, for among a considerable number of such cases we have but one with a distinct metastatic nodule in the kidney (7572), although several cases show extension from retroperitoneal metastases into the capsule, and then into the kidney. On the other hand, the kidneys exhibit extensive diffuse infiltration in leukemia and massive perivascular growths in pseudoleukemia in mice as in man.

The malignant retroperitoneal growths, most of which resemble lymphosarcoma, and the malignant tumors derived from the adrenals, commonly invade the kidney by direct extension into the hilum, often very extensively. The sarcomas primary in one kidney also tend to spread into the hilum of the opposite kidney. This ready invasion of the hilum of the kidney is a point of similarity of human and mouse neoplasms.

No instance of tumor metastasis into the adrenal has ever been observed, except possibly in the few cases of widespread mesotheliomatous growths invading both the ovaries and adrenals, the origin of which is uncertain.

SUMMARY

In a series of 33,000 autopsies on mice of the Slye stock, dying natural deaths at all ages, but as far as possible living out their natural span of life, there have been observed the following cases of true primary neoplasm arising from renal or adrenal tissues: First, from the kidney, 16 tumors, classified as follows: 1 carcinoma, 3 adenomas, 1 hypernephroma, 7 sarcomas, 3 mesotheliomas, and 1 sarcoma of the renal pelvis. Second, from the adrenal, 4 tumors, as follows: 1 cortical adenoma from a misplaced inter-renal adrenal rest, 3 mesothelial tumors. Third, five cases of tumors of the mesothelial structure characteristic of urogenital anlage neoplasms, but of which the exact origin could not be determined because of their widespread growth at the time of death. As these 25 tumors occurred in 33,000 mice presenting not far from 5000 other tumors, they are evidently uncommon tumors of mice, at least in this particular stock.

It will be noted that in this series there has been no instance of a mixed renal tumor of the Wilms type, which is so common a type of renal tumor in man and apparently also in swine. Although inflammatory conditions are very prevalent in the kidneys of mice, epithelial tumors are rare, and especially to be noted is the absence of even a single case of typical malignant hypernephroma, although one benign growth of this type was found. No epithelial tumors of the renal pelvis were found, although there was one case of sarcoma that seemed to take its origin in the pelvis.

Several instances of malignant retroperitoneal tumors have been observed, mostly of sarcomatous structure, which usually invade the kidney. These have not been included in this series, except two cases in which the structure resembled that of the mesotheliomas, suggesting that the tumor had its origin in misplaced rests of the urogenital anlage.

Secondary tumors have never been found in the adrenals, and but rarely in the kidneys. Although this series includes at least 3000 cases of mammary carcinoma, often with widespread metastases in the lungs, we have never seen a secondary carcinomatous growth in the kidney. The only secondary carcinomas of the kidney as yet observed are four cases in which the primary carcinoma was in the lung, thus establishing the true neoplastic nature of these lung growths. In but two cases have metastatic sarcomas been seen in the kidney, if we exclude the numerous cases of invasion of the kidney by direct extension from para-renal growths.

As to sex: In the entire group of renal and adrenal tumors, we have equal numbers in males and females, agreeing with the observation made on other tumors in mice that, in tumors not peculiar to the sex glands, there is usually little difference in the incidence in the two sexes.

Differing from the tumors previously studied, coincidence of other tumors with the renal and adrenal tumors is uncommon. One mouse in this series had a spindle-cell sarcoma of the thigh. One mouse had a small, benign papillary adenoma of the lung. Only two mice had a mammary carcinoma, and one of these (21663) was a remarkable case, for this animal, when but one month old, was found with two independent mammary carcinomas, and with osteosarcomas in the spinal column and in a rib. It lived eighteen days more, and at autopsy there was also found a mesotheliomatous type of growth involving both kidneys. Except for this unique case there have been practically no instances of malignant tumors in mice less than four months of age, and few under six months. Most of the renal sarcomas occurred between the ages of seven months and one year, which is somewhat earlier than the usual time of appearance of epithelial growths; this, of course, corresponds to experience with human neoplasms.

The epithelial renal and adrenal tumors furnished no illustration of metastasis, but in three cases of sarcomatous or mesotheliomatous growths there was noted involvement of the adjacent lymph-nodes; in two there were pulmonary, in two hepatic, and in one splenic metastasis, and in one case there were numerous peritoneal growths. The mesothelial type of growths produced the most extensive metastasis and the most widespread infiltration of the body wall.

A review of the literature on renal tumors throughout the animal kingdom, which constitutes the introductory part of this article, discloses but six other cases of renal tumors in mice, all epithelial, and no other adrenal tumors.

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A CRITICAL INVESTIGATION OF THE FREUND-KAMINER REACTION

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Human tumors

In 1910, E. Freund and G. Kaminer (1) described certain phenomena occurring when tumor cells are mixed with the blood serum from non-cancerous individuals, on the one hand, or blood serum from cancer patients on the other hand. This relationship became known as the Freund-Kaminer reaction. These investigators used tumors obtained at autopsy, because they found it harder to make cell emulsions with fresh material. The healthy portions of the tumor were minced, put into a cloth and pressed out by hand in water containing 0.6 per cent NaCl plus 1 per cent NaFl; the connective tissue and blood-vessels remained behind and the cells escaped. The resulting emulsion was centrifuged and the cells were then washed two or three times with 0.6 per cent saline solution. The washed cells were finally suspended in an equal amount of 0.6 per cent saline solution, and enough NaFl was added to make the solution 1 per cent NaFl; this proportion of NaFl will preserve the cells for three weeks. In many cases the cells agglutinated during washing, so that one washing had to suffice. Organ emulsions were prepared in the same way, and freed of blood by washing once with distilled water.

The serum was obtained sometimes fresh, sometimes post-mortem; sometimes, in later experiments, one-tenth of its volume of 0.5 per cent NaFl solution was added as a preservative.

To perform the test, ten drops of serum and one drop of cell emulsion were mixed, and a drop of 0.5 per cent NaFl solution added; the whole was then well mixed, and the cells in one drop of this emulsion were counted in a Thoma-Zeiss counting chamber. The mixture was then incubated in a test tube with a rubber top at from 37°C. to 40°C., and a second count made. When there were about 20 cells to a large square, their enumeration was easy; more than this were difficult to count, and a mixture with 40 cells to the square, which gave a negative result, gave a positive result with 20 cells to the square.

Freund and Kaminer found that the serum from noncancerous subjects destroys most of the cancer cells. The experiment was controlled by incubating an emulsion of cancer cells in a corresponding amount of 0.6 per cent saline solution containing 0.3 per cent NaFl; no diminution in the number of cells was found. Non-cancerous serum did not destroy normal cells, whether these were from normal persons or from those having cancer. Heating the serum to 55°C. abolished its power to destroy cancer cells. The authors concluded that the serum of non-cancerous individuals must contain a coctolabile substance with carcinolytic powers.

In other emulsions similarly prepared, except that the serum came from cancer patients, there was no diminution in the cell content. All tests were made with the cancer cells of one patient and sera of others.

Freund and Kaminer concluded with the question: "Is the destruction of carcinoma cells by normal serum due to some property in the serum, or to some special fragility of the cancer cell itself?" and they argued that the resistance of carcinoma cells to carcinoma serum points to some lytic property in normal serum rather than to a vulnerability of the carcinoma cell.

Freund and Kaminer's paper aroused great interest, because of the diagnostic possibilities of their reaction, and the experiment was repeated by many other investigators. Thus P. v. Monakow (2) found lysis absent in 86 per cent of cases of carcinoma. A cell destruction up to 10 per cent he considered negative; between 10 per cent to 25 per cent doubtful; and above

25 per cent positive. He observed that besides cancer cells the emulsions contain connective tissue cells, which are not destroyed. In his hands only three-fourths of normal sera caused lysis of cancer cells; one quarter of the normal sera tested acted like cancer sera and one-fifth of all cancer sera destroyed cancer cells like normal sera. He, as well as Freund and Kraus, found that the reaction often failed in sarcoma.

Stammler (3) concluded that the Freund-Kaminer test showed 80 per cent positive reactions.

Ranzi (4), in 1911, in a review of diagnostic reactions for malignant tumors, concluded that the serum of cancer patients contains a substance protective for cancer cells.

Kraus and v. Graff (5) attacked the problem from a different angle. They knew from the work of Salomon and Saxl that both in pregnancy and in carcinoma the excretion of oxyprotein is increased, and that the polypeptids are increased in carcinoma and in the later months of pregnancy. On the assumption of some analogy between the two conditions, they tested the serum of pregnant women and serum from the umbilical cord, and found that cancer serum as well as that from the umbilical cord failed to dissolve cancer cells. The serum of pregnancy caused lysis of cancer cells only inconstantly, while serum drawn after confinement had no lytic action. They concluded that the placenta produces alterations in the serum causing a loss of carcinolytic qualities or the formation of a protective substance. Rabbit and guinea pig serum acts as normal human serum, and rat, goat, and sheep serum act as carcinoma serum, the former being lytic and the latter not.

Kraus, v. Graff, and Ranzi (6) also tested the reaction, and found that 71.4 per cent of tumor patients examined gave a positive reaction (lysis below 25 per cent) and 3.5 per cent a partial lysis. Of patients with other diseases and with benign tumors, 61.2 per cent gave a negative reaction, 15.3 per cent a positive reaction, and 23 per cent a partial lysis. Seven cases that had been operated on and were clinically free from recurrence gave a negative reaction (lysis over 50 per cent); hence they concluded that the Freund-Kaminer reaction is not based on an acquired tumor disposition but is due to changes in the blood serum depending upon metabolic changes in the tumor.

Kraus and Ischiwara (7) tested embryonal cells against cancer cells, and found that human embryonal cells show even greater lysis with normal serum than do cancer cells. Furthermore, embryonal cells were cytolyzed by a cancer serum which had no action on cancer cells. There was no lysis with fetal blood serum; but maternal (retroplacental) serum was lytic.

Rosenthal (8) continued along these general lines, using a 25 per cent emulsion of cells suspended in physiological saline solution as a control. He believed that only a lysis exceeding 25 per cent can be ascribed to the action of serum. His results were as follows: Fetal cells plus pregnancy serum gave a lysis beyond 25 per cent. Fetal cells plus fetal serum showed a lysis beyond 25 per cent. Fetal cells plus umbilical cord serum gave no cytolysis.

Arzt and Kerl (9) also have attempted to determine the diagnostic value of the Freund-Kaminer reaction. They found in the group of tumors examined 83 per cent correct diagnoses and 17 per cent wrong. These diagnoses were verified by biopsy, by operation, or by autopsy. In healthy persons and in those suffering from non-tumorous diseases the reaction gave 87.5 per cent correct and 12.5 per cent incorrect diagnoses. Freund and Kaminer, when consulted about these figures, suggested that the wrong diagnoses might be due to such errors in technique as: (*a*) obtaining blood during the height of digestion; (*b*) admixture of red blood cells with the serum; (*c*) the use of insufficiently fresh serum; (*d*) the use of cells that had lost their sensitiveness.

Kraus, Ischiwara, and Winternitz (10) reached the decision that human serum can destroy cancer cells but not normal cells, and that embryonal cells behave as cancer cells in relation to adult and umbilical cord serum.

This work attracted the attention of Coca (11) who carried out more than 150 experiments, some of them in the laboratory of Freund in Vienna. All his experiments, however, even those performed in Freund's laboratory, resulted negatively, no cytolysis being observed in any instance with fresh normal human, dog, or horse serum. He believed, therefore, that the cytolytic action of normal sera described by Freund and Kaminer depended upon some factor as yet uncontrollable, and that it cannot be made the basis of a differential test for malignant tumors.

Freund and Kaminer have recently published (12) further experiments, to show that normal serum and tissues contain an organic fatty acid able to destroy cancer cells; this they call "normal acid." Cancer and the serum of cancer patients contain an acid that protects the cancer cell by destroying the normal acid. Pending an identification of the "normal acid," an investigation was undertaken of the saturated dibasic fatty acids, the series to which they believe "normal acid" belongs, in order to see which ones are able to destroy cancer cells.

The most recent publication is that of Frankenthal (13), who found lysis of cancer cells treated with normal serum to beyond 50 per cent; but no lysis of cancer cells treated with the serum of fourteen patients having non-cancerous diseases; 25 sera of cancer patients gave 7 (28 per cent) negative results. Her conclusions are: (*a*) normal serum destroys cancer cells, but also normal liver cells in lesser degree; (*b*) cancer serum acts differently from normal serum, in that it protects the cells from destruction up to a certain degree; (*c*) the Freund-Kaminer reaction is neither constant nor specific and, therefore, of no diagnostic value for the present.

Animal tumors

Freund and Kaminer used tumors (as stated above) that had been in the mortuary or the laboratory for indefinite periods of time, and it is open to speculation what proportion of the cancer cells so used were alive. They also state that the serum used in their experiments was sometimes obtained fresh and sometimes postmortem, and one may justly ask whether this serum might not have been more or less decomposed. Hirschfeld and Ischiwara, working independently of each other, apparently saw this objection, for they used freshly drawn animal serum.

Animal tumors also have been employed, their great advantage being, of course that the viability of their cells can be tested by inoculation. Thus Hirschfeld (14) made a very fine emulsion of tumor cells, exposed it to the serum of non-tumor bearing animals or to that of rats with sarcoma in the proportion of 1 to 6, incubated it for three hours, shaking the mixture every twenty minutes; and then diluted it with equal parts of 0.6 per cent saline. One cubic centimeter of this suspension was inoculated into the right inguinal region of normal animals, and a series of control animals was inoculated with tumor emulsion in physiological saline solution. The tumors in the animals inoculated with tumor cells treated with normal serum were fewer and smaller than in those inoculated with tumor cells treated with tumor serum. He concluded that this experiment proves again that normal serum has a damaging action on the vitality of tumor cells, while the serum of tumor-bearing animals lacks this quality.

Finally, Ischiwara (15), working with rat sarcoma demonstrated that the cell reaction appears relatively late (thirty days) in the serum of rats, and has some relation to the size of the tumor.

The reaction has been tested from the refractometric side by Koritschoner and Morgenstern (16), and by Koritschoner alone (17).

EXPERIMENTAL

In retesting Freund and Kaminer's experimental conclusions the present writer has also retested Kraus and v. Graff's observation (5) that the serum obtained from rabbits and guinea-pigs acts on cancer cells as does normal serum; i.e., causes lysis, whereas that from rats, goats, and sheep acts like carcinoma or placenta serum, i.e., has no appreciable lytic effect. If this were proved to be correct, then the conclusions drawn from all the experiments in which normal rat serum was used (Hirschfeld and Ischiwara) would obviously have to be considered erroneous.

In a series of experiments in which blood was drawn from the heart of rats and guinea-pigs, and from an ear vein of rabbits, this observation was tested in the following manner.

The blood, drawn under aseptic precautions, was centrifuged and the resulting serum was pipetted off, care being taken not to allow any admixture of red blood cells, since Freund, in a discussion of reported failures had attributed them to faulty technique. As another suggested error was the use of serum not sufficiently fresh, all sera obtained were used immediately. Freund also attributed failure to the use of blood obtained at the height of digestion, and to avoid this the experiments now being described were made with animals which had not been recently fed.

The tumor used in making the suspension was the Flexner-Jobling rat carcinoma, since all other experiments along these lines had been carried out with rat sarcoma in spite of v. Monakow's observation, corroborated by Freund and Kaminer, that the reaction often fails with sarcoma. The tumors selected were from thirty to seventy days old, it being generally believed that transplantability and growth capacity increase, in a general way, with the age of the tumor. In no case was the skin of the animal attached to the tumor, nor were any of the growths ulcerated. Furthermore if an animal appeared sick its tumor was not used.

The tumors were extirpated under aseptic precautions and the connective tissue capsule was carefully removed, for previous observers had stated that the admixture of connective tissue vitiated the test. Thus the only connective tissue remaining was the small amount comprising the stroma.

Only the healthy margin of each tumor was used and microscopic sections were made for purposes of checking up each experiment.

A fine emulsion of the tumor was then made by thoroughly mincing it with scissors, in order that the serum might come into contact with all parts of the growth. Various machines devised for emulsifying tumors were at first used but it was found that the resultant particles were too coarse and that a much finer emulsion could be obtained by using very sharp, curved manicure scissors. All manipulations, of course, were conducted under strictly aseptic precautions.

This fine tumor emulsion was next added to the serum in the proportion of 1 to 6, and the suspension was placed in test tubes and incubated at 37°C., for three hours. Some fresh tumor emulsion was immediately transplanted, without exposure to serum, into a series of healthy laboratory rats known to be susceptible to the Flexner-Jobling carcinoma, in order to prove the viability of the tumor employed. The amount transplanted was 0.003 gram fresh tumor emulsion, which was deposited in the right inguinal region with the usual inoculating needle. The tubes placed in the incubator were each supplied with a sterile glass rod and the emulsion was vigorously stirred every half hour in order to bring all particles of the tumor emulsion thoroughly into contact with the serum and so allow the serum to exert its influence on the largest possible number of tumor cells. At the end of the three-hour period all supernatant serum was poured off and the fine particles transplanted into a series of rats.

The protocols of a few experiments selected at random from a considerable number follow:

Comparison of the number and size of the tumors in figure 1 shows no distinct difference between the control tumors and those exposed to rabbit serum. In the serum series there is found one receding tumor; but recession of the Flexner-Jobling carcinoma is a common event, and does not vitiate the results of this experiment. One inoculation was negative, but only about 80 per cent of inoculated animals, under routine conditions, grow this tumor. One animal died before the first charting. On the whole, one may say that the animals in both series had tumors of from 10 mm. to 40 mm. in diameter and that the takes are entirely comparable in both series.

Reference to figure 2 shows that in both series after forty-five days there are tumors from 10 mm. to 40 mm. in diameter, the number of growing tumors being practically 100 per cent, with the exception of one receding tumor in the serum series.

It is evident from figure 3 that the tumors were more numerous as well as larger in the serum series than in the controls.

In the next group of experiments the effect of incubation in guinea-pig serum was studied.

As may be seen from figure 4, 45 days following the inoculation there were 100 per cent takes in both series, and there is no obvious difference between the results in the two groups.

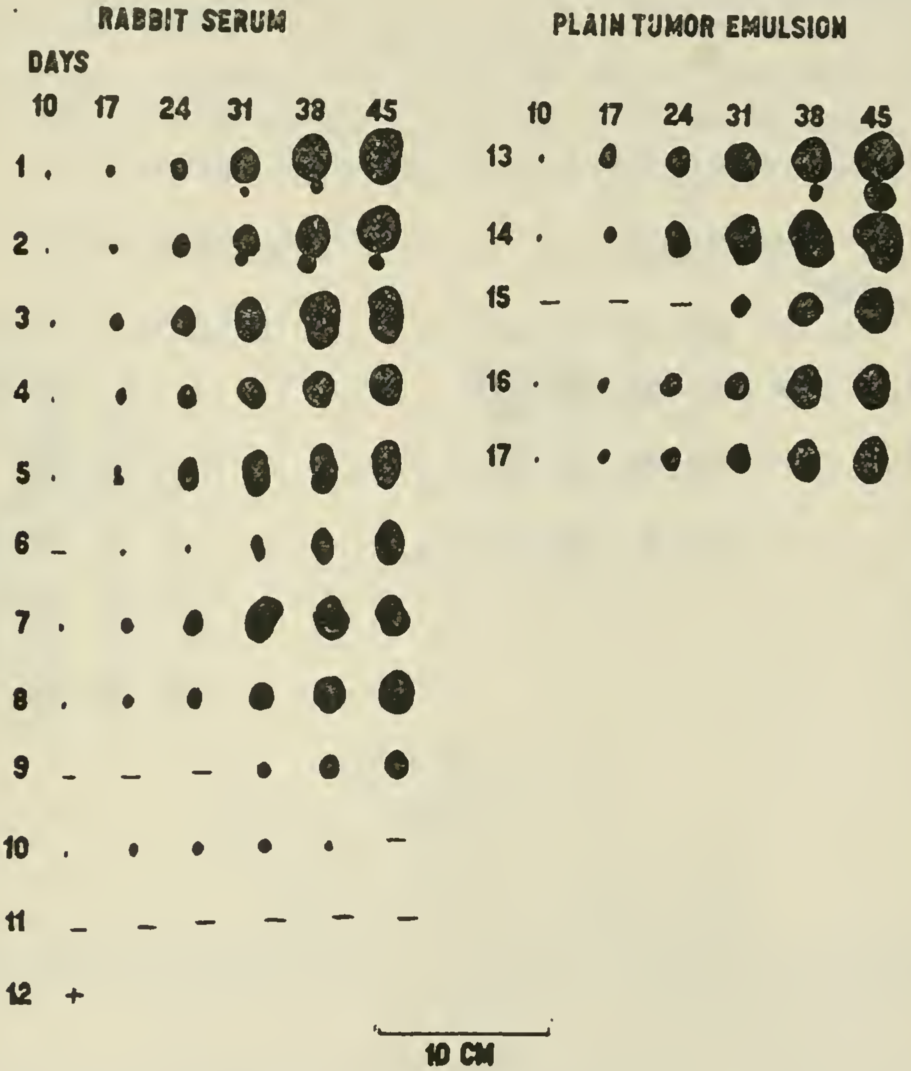

Fig. 1. Experiment $\frac{\text{F R C}}{\text{89 A}}$

An emulsion of the Flexner-Jobling rat carcinoma, diluted with five parts of rabbit serum, was incubated for three hours and then transplanted into a series of 12 rats (nos. 1 to 12). Fresh tumor emulsion was inoculated into a series of 5 rats (nos. 13 to 17) to act as a control.

Reference to figure 5 will prove that there are 80 per cent takes in the serum group, whereas in the fresh tumor, or control series, there are only 40 per cent takes, and the tumors are actually smaller. Clearly the guinea-pig serum exerted no deleterious effect on the tumor cells.

It can be seen from figure 6 that forty-two days after the inoculation there were 100 per cent takes in both series and there is no obvious difference that can be observed in comparing the results in the two series.

Rabbit and guinea-pig serum may have a different action on human cells, but for the purpose of the present investigation the road is cleared by the proof that the serum from neither of these species has a deleterious effect on the tumor cells employed.

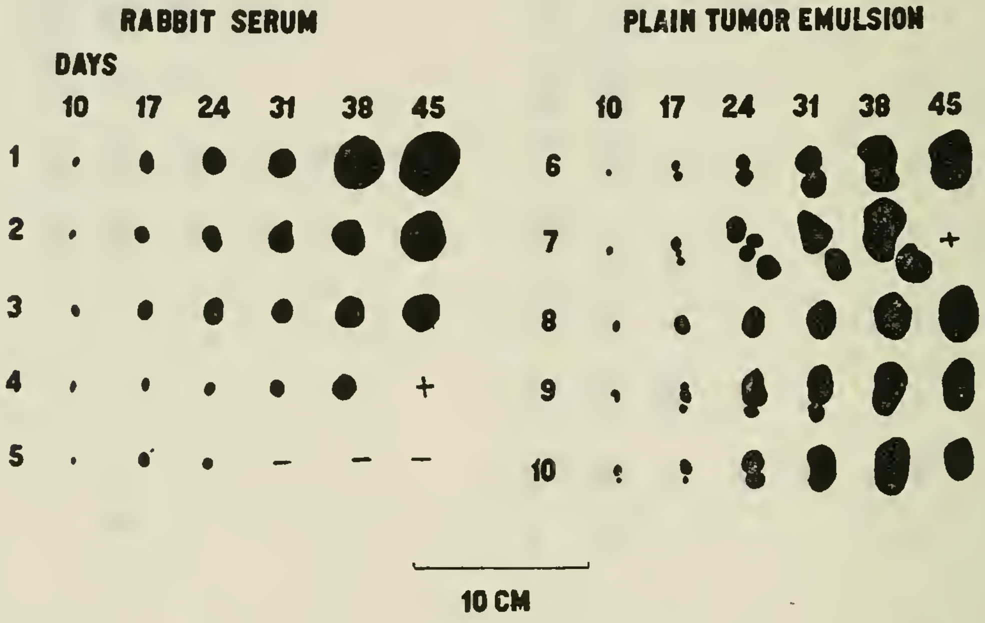

Fig. 2. Experiment $\frac{\text{F R C}}{\text{89 E}}$

Five rats (nos. 1 to 5) were inoculated with an emulsion of the Flexner-Jobling rat carcinoma incubated in rabbit serum in the proportion of 1 to 6, and 5 rats (nos. 6 to 10) with fresh tumor emulsion.

The results of these experiments do not agree with Kraus and v. Graff's observation that the serum obtained from rabbits and guinea-pigs exerts a damaging action on cancer cells; for, in some instances (figs. 3 and 5) the cancer cells subjected to the action of serum from these animals grew even better than did cells from the same tumor which had not been subjected to the action of the serum. We do not attempt to explain the reason why previous observers have obtained such different results.

The objections to Freund and Kaminer's method have been already discussed. To obviate these, a series of experiments was carried out on animal tumors because here both cells and serum can be obtained perfectly fresh, and the viability of the tumor cell can be tested by inoculation. Cells from rapidly growing rat carcinomata (Flexner-Jobling) were suspended in the serum

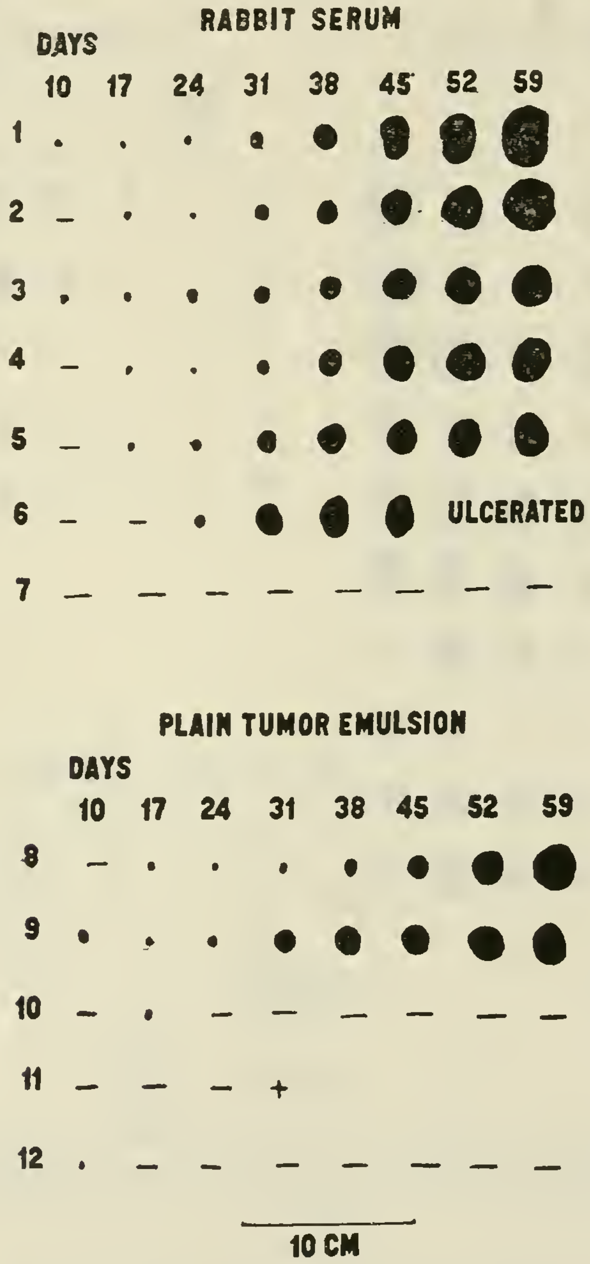

Fig. 3. Experiment $\frac{\text{F R C}}{\text{90 K}}$

A series of rats (nos. 1 to 7) was inoculated with an emulsion of the same tumor incubated in rabbit serum in the proportion of 1 to 6, and 5 rats (nos. 8 to 12) with fresh tumor emulsion to serve as a control.

of normal rats, or of rats bearing this tumor, without the intervention of any extraneous substance such as NaCl or NaFl. It was realized, of course, that only the first half of the experiment is really valid, since the serum of an animal with a transplanted tumor may be entirely different from that of one with a spontaneous neoplasm. But the extreme rarity of carcinoma in the rat, and the impossibility of using a sarcoma left no other course open. Mouse carcinoma was not used because of the scanty amount of serum obtainable from so small an animal.

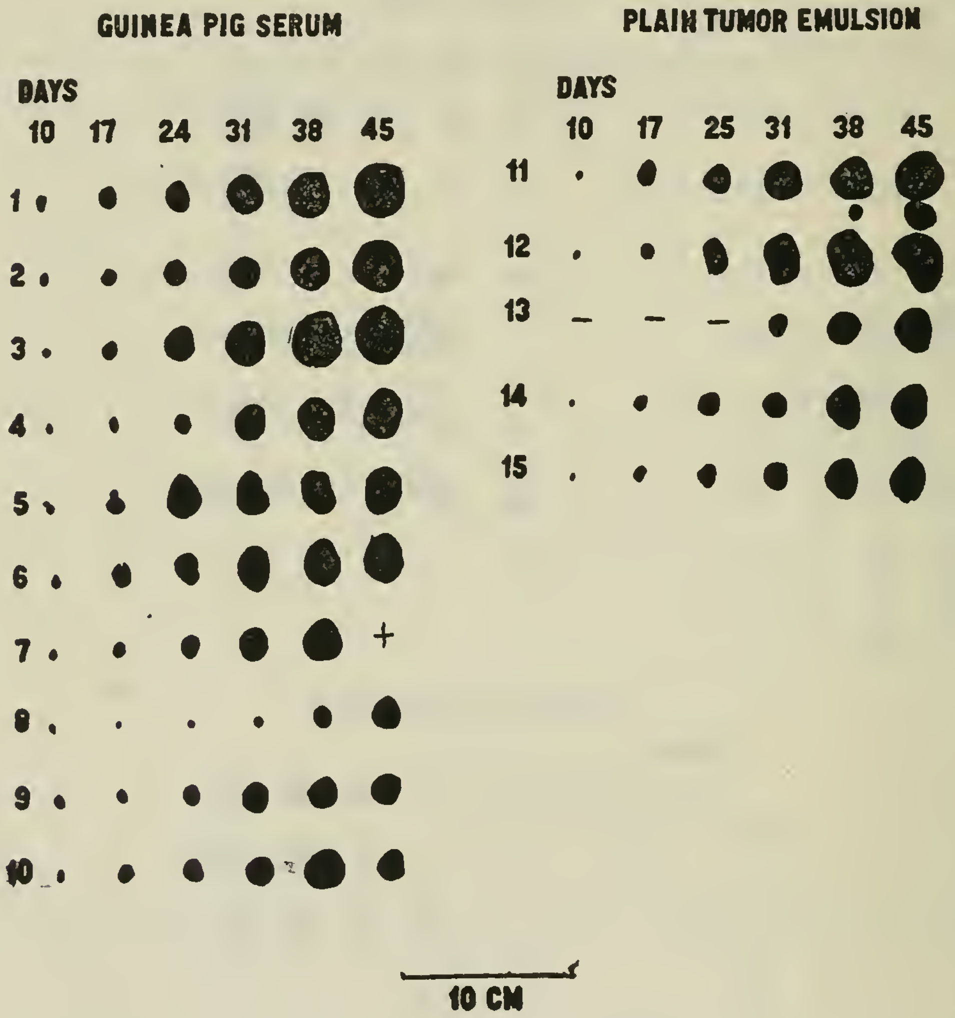

Fig. 4. Experiment $\frac{\text{F R C}}{\text{89 A}}$

A series of 10 rats (nos. 1 to 10) was inoculated with an emulsion of the Flexner-Jobling rat carcinoma incubated in guinea-pig serum in the proportion of 1 to 6, and a series of 5 rats (nos. 11 to 15) with fresh tumor emulsion for controls.

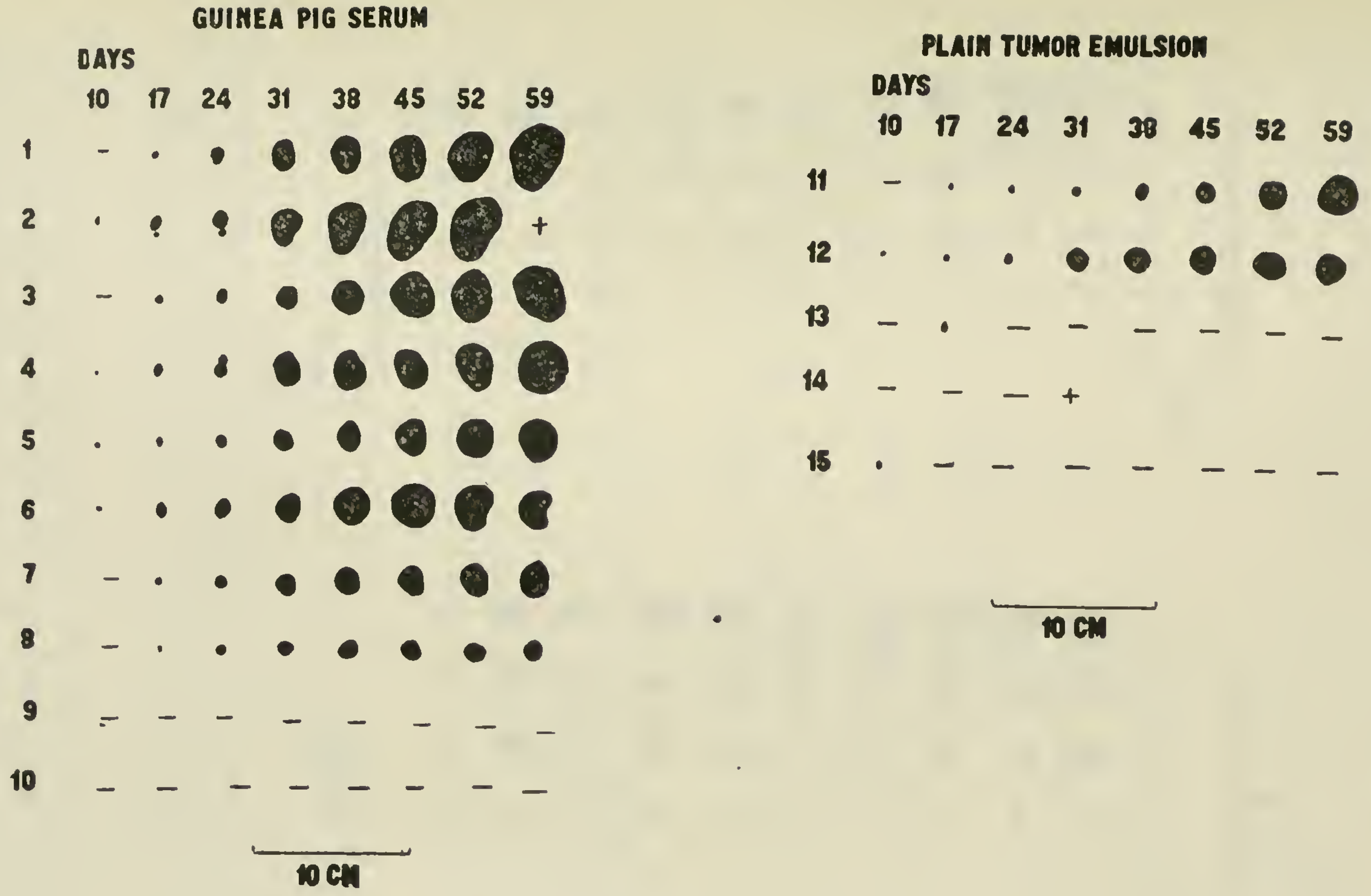

Fig. 5. Experiment $\frac{\text{F R C}}{\text{90 K}}$

A series of 10 rats (nos. 1 to 10) was inoculated with an emulsion of the same tumor incubated in guinea-pig serum in the proportion of 1 to 6, and a series of 5 control rats (nos. 11 to 15) with fresh tumor emulsion.

Nevertheless, it can be shown whether or not normal serum damages the tumor cell; if it does not, the distinction between the serum of a normal animal and of one with a spontaneous tumor vanishes.

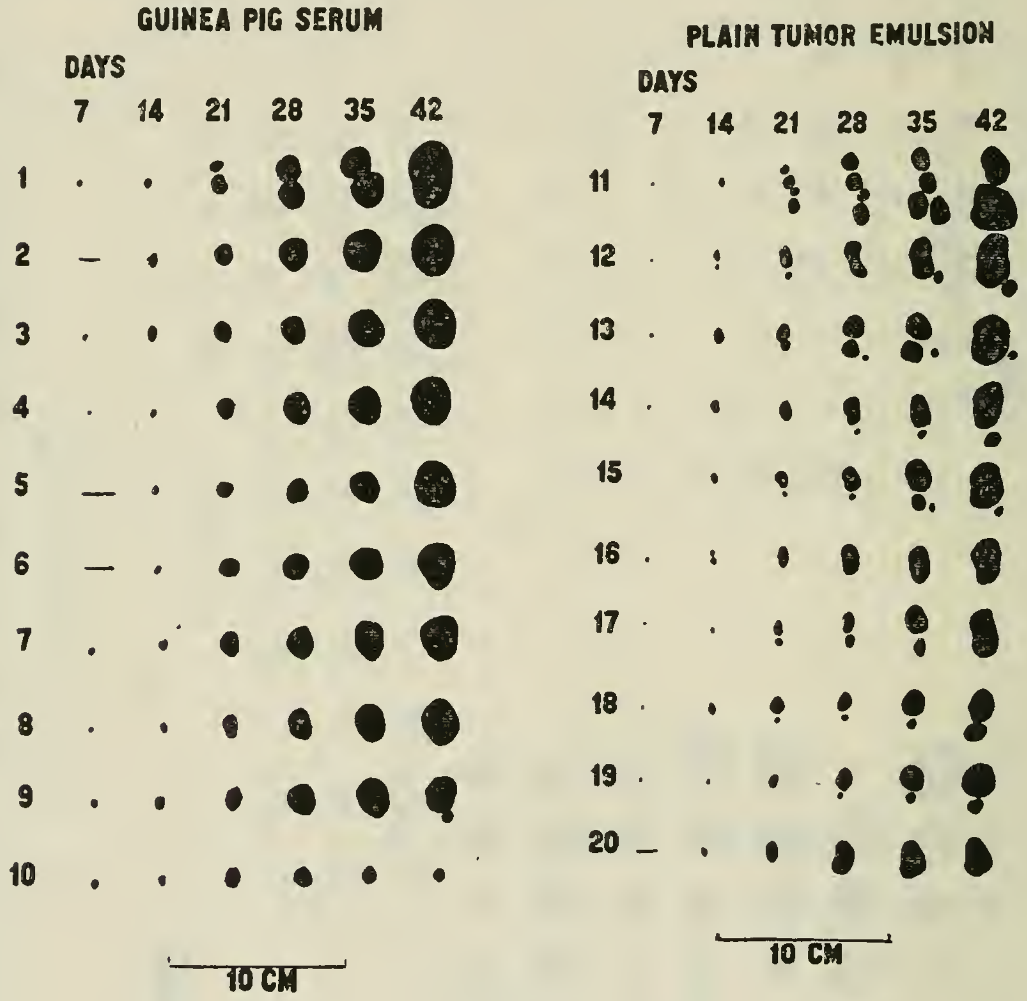

Fig. 6 Experiment $\frac{\text{F R C}}{\text{92 B}}$

A series of rats (nos. 1 to 10) was inoculated with an emulsion of the Flexner-Jobling rat carcinoma incubated in guinea-pig serum in the proportion of 1, to 6, and a series of 10 rats (nos. 11 to 20) with fresh tumor emulsion for controls.

The experiments were carried out exactly as were those with rabbit and guinea-pig serum. The blood was obtained from the hearts of rats bearing Flexner-Jobling tumors from four to eight weeks old. All animals had large single or multiple tumors, ranging from 20 mm. to 35 mm. in diameter, none of which were fixed to the skin or ulcerated. Nor did any of these animals manifest any evidence of illness.

While the other manipulations necessary to the experiment were conducted, the serum was placed in the refrigerator at 8°C. Similarly a number of rats without tumors were bled and the serum also placed in the refrigerator.

A healthy tumor was chosen, its capsule carefully stripped off, any necrotic material thoroughly scraped out, and the outer layer of the remaining healthy tumor tissue finely emulsified. All tumors used in these experiments were from thirty to ninety days old and microscopic sections of each tumor used were made and filed away for purposes of identification.

The sera were now taken from the refrigerator and to each was added this finely minced tumor in the proportion of 1 part of tumor to 5 parts of serum. A sterile glass stirring rod was added to each tube, and the tubes were incubated at 40°C. for three hours. Every half hour each tube was stirred with its glass rod in order to bring all tumor cells repeatedly into contact with the serum.

At the end of the incubation period all the serum was poured off and the fine tumor particles were transplanted into a series of rats in the manner previously described. A similar dose (0.003 gram) of fresh tumor emulsion was transplanted in the same way and at the same site without its having been exposed to any serum.

The protocols of a few typical experiments follow:

Figure 7 shows that the tumor cells incubated in normal serum grew better and more uniformly than did those incubated in tumor serum, and even better than the untreated tumor emulsion which had not been in contact with any serum whatsoever.

Figure 8 shows again that there was better growth in the series of animals inoculated with cancer cells incubated in normal serum, than in the tumor serum series. The results in the untreated tumor emulsion series were similar to those obtained in the normal serum series.

After thirty-eight days, as figure 9 shows, the results in all three series were practically alike, the tumors varying in diameter between 7 mm. and 20 mm. and growth being about the same.

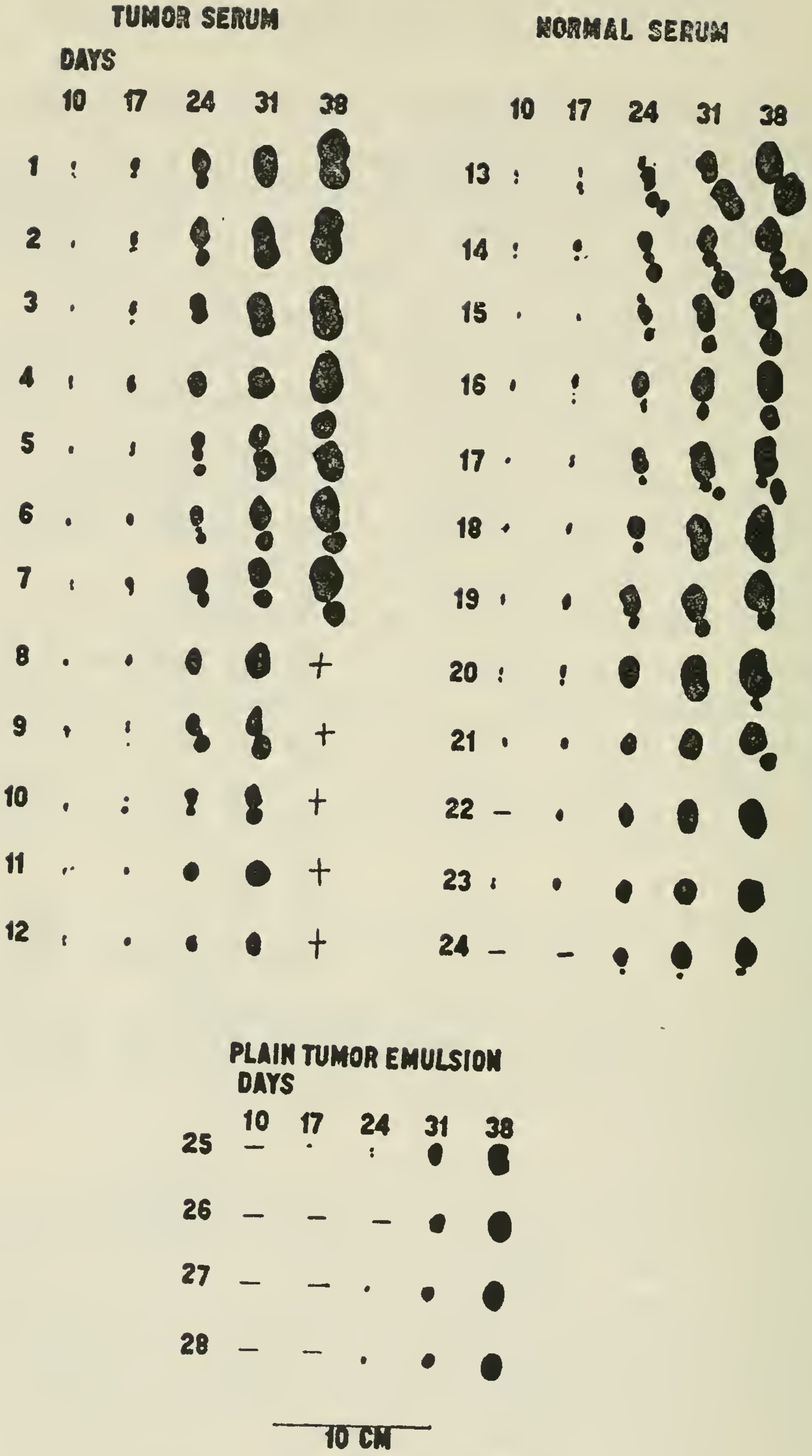

Fig. 7 Experiment $\frac{\text{F R C}}{\text{83 B}}$

An emulsion diluted in the proportion of 1 to 6 with tumor serum, and in the same proportion with normal serum, was incubated and then transplanted into two series of animals, twelve each in number. Into a series of five animals a fresh undiluted tumor emulsion was transplanted.

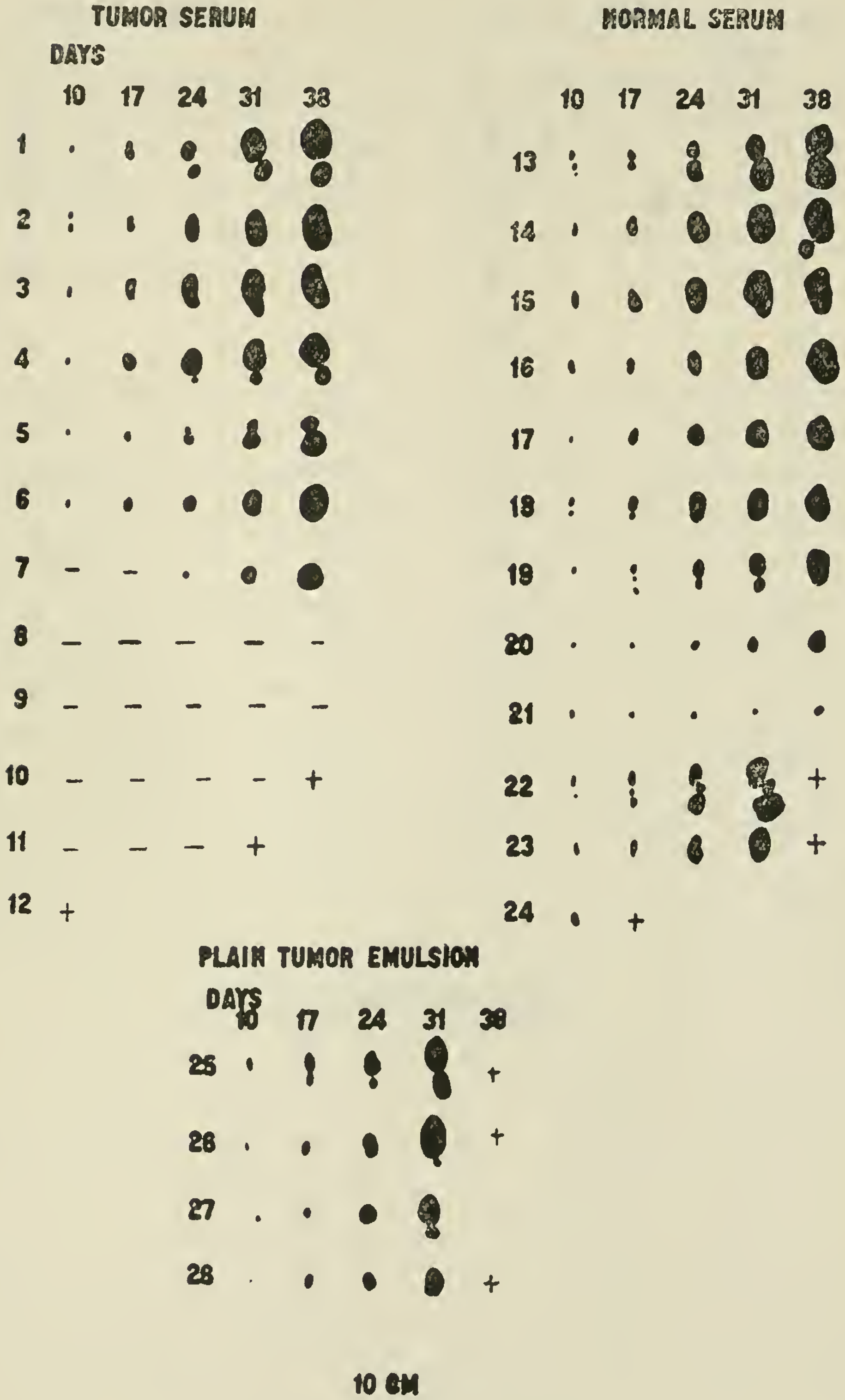

Fig. 8 Experiment $\frac{\text{F R C}}{\text{84 N}}$

An emulsion diluted in proportion of 1 to 6 respectively with tumor serum and normal serum was incubated for three hours and then inoculated into two series of twelve animals each and the fresh tumor emulsion into a series of five animals.

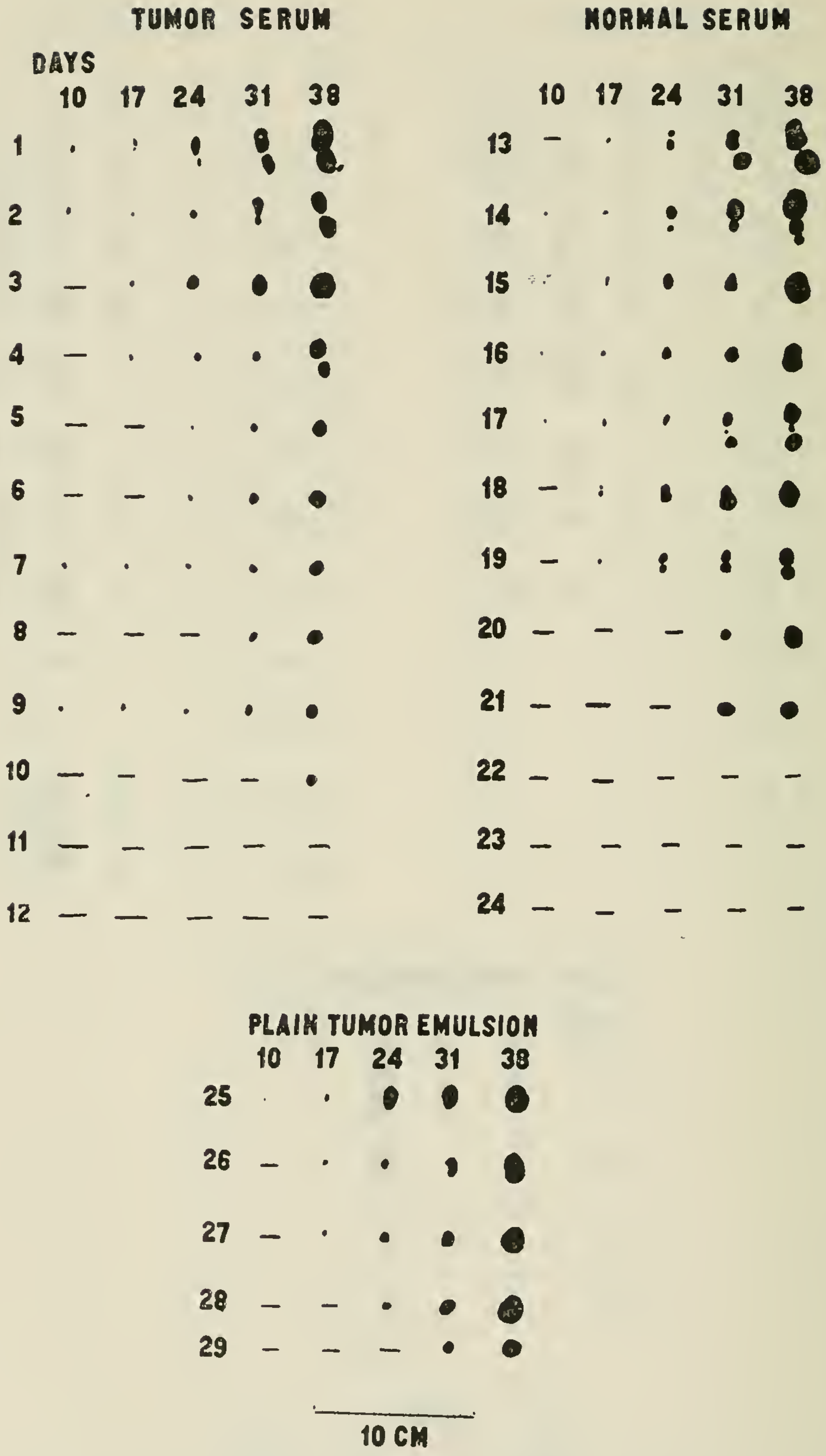

Fig. 9. Experiment $\frac{\text{F R C}}{\text{85 D}}$

An emulsion diluted in the proportion of 1 to 6 respectively with tumor serum and normal serum was incubated for three hours and then inoculated into two series of twelve animals each and a fresh tumor emulsion into a series of 5 animals.

Obviously the serum of normal rats exerts no deleterious influence upon the cells of the Flexner-Jobling carcinoma, for in three separate experiments cells incubated in normal serum grew quite as well as those incubated in serum from rats with transplanted tumors. Nor was there any difference in growth between cells exposed to either serum and those transplanted without previous manipulation.

CONCLUSIONS

The conclusions to be drawn from the whole series of experiments are equally clear. After having made sure that there was no difference in the action of either rabbit or guinea-pig serum on the one hand, and rat serum on the other; and after having eliminated the sources of error enumerated by Freund and Kaminer, namely the admixture of connective tissue capsule and its blood-vessels, the presence of red blood cells in the serum, the use of old serum or of serum obtained at the height of digestion; and having, furthermore, used carcinoma (the most sensitive tumor to this test, according to the originators) instead of sarcoma, as had been done by previous investigators, we are still unable to detect any marked difference in the results of inoculation whether tumor serum or normal serum is used. The growth capacity seems neither increased in the one nor diminished in the other. We feel justified in stating, therefore, that the value of the Freund-Kaminer reaction remains at present unproved. With this proviso, however, that these experiments prove only that the serum of normal rats is devoid of any deleterious effect on the Flexner-Jobling rat carcinoma. What might be the case with serum from a rat bearing a spontaneous carcinoma, we do not yet know. However, the first half of the experiment seems sufficient; for since this shows clearly that normal serum has no harmful effect upon the cancer cell, there can be no difference, with respect to cytolytic power, between normal serum and that of an animal with a tumor, be this transplanted or spontaneous.

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IS CANCER A BIOLOGICAL PHENOMENON?[1]

SOME HERETIC THOUGHTS ON CANCER

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The primary causes of cancer are still unknown. It seems, however, more and more evident that the development of cancer is particularly favoured by certain conditions, viz. chronic local irritants and old age.

The *chronic local irritants* are of the most variegated descriptions, belonging to all manifestations of energy—mechanic, thermic, chemical, radiological; there are also vital (animal) irritants (Bilharzia, Spiroptera neoplastica s. Gongylonema neoplasticum, Fibiger), and pathological irritants (precancerous conditions).

But the very difference in the nature of all these irritants indicates that it is scarcely the irritants themselves but more likely the reaction of the organism against these, which offers the conditions favourable for the development of cancer. Borrmann and Ribbert emphasize that, previous to the cancerous proliferation of the epithelium there is constantly found an inflammatory alteration of the underlying connective tissue, representing an alteration of the conditions of life of the epithelial cells.

Very frequently, though, cancer develops in localities where no irritants or irritation have been noticed.

The *influence of age* is more obvious; statistics from all countries here agree. In Norway, the mortality from malignant tumors, for the years 1901–1915 is shown in table 1.

Of these 32,274 deaths from malignant tumors, only 206, or 0.64 per cent, have occurred in persons under thirty years of age;

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[1] Paper read in part before the Medical Society of Kristiania, September 21, 1921.

only 1146, or 3.55 per cent, under forty years, though these groups include 59.27 per cent and 71 per cent respectively of the whole population.

It is also remarkable that the maximum of deaths from cancer occurs in the period from sixty to seventy years of age, with nearly 30 per cent (column 6) of all cancer deaths, in spite of this period of life numbering only 5.96 per cent (column 2) of the population. And in the period from seventy to eighty years there is still an increase as to the relative importance of cancer as the cause of death. After the age of eighty years there seems to be some decline in the relative cancer mortality; but this is without doubt only apparent, numerous deaths from cancer in the latest decenniums of life being covered by the diagnosis of senile debility as well as by other intercurrent causes of death.

TABLE 1

Cancer mortality in Norway, 1901–1915, distributed in decennial groups

(1)	(2)	(3)	(4)	(5)	(6)	(7)
AGE	POPULATION OF NORWAY 1910		CANCER MORTALITY IN NORWAY			
	Number of individuals	Per hundred of the population	Total from 1901–1915	Average yearly	Per hundred of all cancer deaths	Yearly per 100,000 living
years						
1–20	1,063,262	45.10	38	2.53	0.12	0.024
20–30	334,091	14.17	168	11.20	0.52	0.34
30–40	275,800	11.69	940	62.66	2.91	2.27
40–50	225,181	9.56	3,226	215.06	9.99	9.55
50–60	194,109	8.24	6,925	461.66	21.45	23.73
60–70	140,579	5.96	9,658	634.85	29.93	45.80
70–80	83,825	3.56	8,462	564.15	26.22	67.30
80–90	32,551	1.38	2,716	181.00	8.41	55.60
90 and more.............	3,065	0.13	141	9.4	0.45	30.67
No age reported.........	5,327	0.19				
Total.................	2,357,790	99.99	32,274	2151	100.00	9.15

Former investigations by the Norwegian Committee for Cancer Research as well as the recent ones by Dr. Jørgen Berner make it very probable that the frequency of cancer continually increases with increasing age.

The table shows that the ages above thirty to forty years are affected by cancer proportionally 226 resp. 66 times as often as the ages below the same years.

Age must consequently be of the greatest importance for the development of cancer, and Bashford's saying, that "cancer is a function of age" contains a great amount of truth.

It must therefore be one of the nearest and most important objects for the cancer research work to investigate this influence of age.

Surely it has been hinted that age just gives the chronic irritant sufficient time to act. But this explanation of the influence of age is hardly satisfying. We do not know the time necessary for any irritant to produce cancer. But suppose that even ten or twenty years are required, there will still be every opportunity for an irritant to unfold its activity in many more thousands of cases in the twenty to forty years group, than in the older groups, but cancer is very rare at the younger age. And some of the irritants, for example, the *x*-ray, seem to be able to produce cancer after a proportionally short time and in younger individuals.

It seems more natural to attribute the influence of age upon the development of cancer to the change of metabolism in the aged. This change alters conditions of life for cells and tissues, and a local irritant may add directly and indirectly to these alterations and accentuate the influence of senile metabolism.

The influence of age on the development of cancer is so pronounced that it seems to signify a biological law. That cancer occasionally may develop in younger individuals makes no difference as to the regular influence of age. It is not the mere number of years of life that constitutes "age", but the individual's physiological condition. This senile condition may be postponed in many aged individuals; on the other side a senilitas praecox can be found in younger individuals, as we see it in the arteriosclerosis with young neurasthenics.

Unfortunately we do not know enough about the metabolism of age to be able to form any precise conception of its influence upon cancer formation. "Geriatry" has not been studied as thoroughly as pediatry.

Amongst the more important somatical peculiarities of age may be mentioned: diminution of the total mass of blood, reduction of produced and needed calories, lower temperature of the body, greater amount of cholesterin in the fat cells, increased total nitrogen in the blood serum, reduction of the number of cells in the connective tissue and of their regenerative power, atrophy of the elastic tissue, of lymph nodes, spleen, Peyer's lymphatic noduli, and of the bone marrow. Then, also, a change and diminution of the functions of the endocrine organs.

The senile changes appear at different times in different individuals as well as in the different organs of the same individual. The senile changes can occupy the entire organism (general senility) or only certain organs or parts of these (local or partial senility). Local senility can be found in the female generative organs (ovaries, uterus, and mammæ), where physiological activity and involution occur years before the manifestation of the individual's general senility.

In accordance with this fact we see that cancer of the female reproductive organs appears earlier in life than cancer in the other organs, the great majority of deaths here occurring between forty and sixty years of age (Norwegian statistics), in contrast to deaths from cancer in other organs, where the greatest numbers are to be found from sixty to eighty years of age, twenty years later. The chorionepithelioma malignum has also its greatest frequency in individuals above forty years of age (Anton Sunde).

At present we may assume that local chronic irritants, and old age, produce the altered conditions of cellular life which entail cancer.

The real nature of these conditions is still unknown to us. But it may be worth remembering, that Freund and Kaminer ten years ago have found in the blood serum and tissue fluids of healthy (non-cancerous) persons a well defined fatty acid ("normal acid"), soluble in ether and having the power of dissolving cancer cells. This acid, that has some resemblance to the decamethylencarbonic acid $(COOH)_2$ $(C_2H_4)_5$, of the succinic acid series, is not to be found in blood or tissue serum from cancerous individuals, whose blood has a higher alkalinity than that of non-cancerous individuals (Maud Menten).

We know from the experiments of Jacques Loeb and others that the division and multiplication of cells can be influenced by very small changes of their surrounding nutritive mediums. Similar facts are known from bacteriology.

Robertson and Burnett have been able to accelerate the growth of cancerous tumors, inoculated upon rats, by injecting the animals subcutaneously with cholesterin (and with tethelin), substances which in themselves do not produce cancer in the said animals. Luden and Bloor have also stated that the blood of cancerous patients contains more cholesterin than normally. That the fat cells in old individuals are richer in cholesterin than those in younger persons has been mentioned above.

Ferd. Blumenthal noticed that the growth of inoculated cancer in rats was accelerated when the animals got a surplus of potassium in their food, but retarded by a surplus of calcium.

Direct and local influences upon the growth of the epithelium are known since the experiments of Fischer and others with Scharlach R (and other similar substances), the application of which on the skin of rabbits produced a lively proliferation of the epithelium. And Yamagiwa and Ischikawa, and now lately Fibiger have through similar application on rats succeeded in producing growth possessing the essential qualities of the genuine carcinoma.

The local irritations and the old-age changes of metabolism entail both altered conditions of life for the cells, consequently also a change of their general manifestation of life.

But such changes in general manifestations of life are just what we find as criteria for the transformation of a normal body cell into a cancer cell: changes in outer shape and structure building power, changes in demand for nourishment, in speed of growth and proliferation, in chemical structure, enzymes and secretions (cachectic power), in sensibility towards electrical influences (x-rays), and independence of soil (metastases).

These changes of essential biological qualities in the cancer cell are just what v. Hansemann has designated under the name of *anaplasia* (transformation), a phenomenon hitherto considered as unique, unparalleled.

But is such a transformation really so unique in the living nature?

About twenty years ago de Vries introduced into biology the phenomenon of *mutation,* the comparatively quick changes of the characteristics of species in living beings, in contrast to the very slow changes admitted by the Darwinistic principles of evolution. This mutation, first noticed by de Vries in a plant (oenothera), has later been described for unicellular organisms, bacteria (Weisser, Massini), algae, spirochaetae, trepanosomas, infusoria, and amebas. Thjøtta and Eide have described a paratyphoid strain that, under culture for about a month, essentially changed its character morphologically and physiologically, loosing its flagelli and motility and changing into a capsulated mucus-forming bacillus; which shape it preserved through all the consecutive generations.

Gurney Dickson, in his monograph on the Transmutation of Bacteria, maintains that mutation is an adaption to altered conditions of life; it can be more or less pronounced and may include very different qualities of the bacteria: shape and size, affinity to dyes (chemical and physical properties of the protoplasm), ability of growing on a new soil, resistance against external influences, virulence, and production of ferments. Most frequently mutation occurs in cultures of old date.

There seems thus to be a *considerable parallelism between mutation and the phenomena accompanying the transformations of a normal body-cell into a cancer cell.* And the mutated cells, like the cancer cell, will transfer the newly acquired qualities to their future generations.

Through all these changes in its fundamental biological qualities the cancer cell has turned out to quite a new cellular being, foreign or even *hostile,* to the organism in which it has developed. Authors like Bashford, Abderhalden, Ribbert, Butlin and others call the cancer cell a parasite. Butlin even described it as a new and special species of beings under the name of *unicellula cancri.*

The idea that the transformation of the normal cell to a cancer cell may be a *mutation,* can certainly not explain the nature of cancer, the nature of mutation being still enigmatical, but it brings the cancer question in a line with other biological phenomena, which may be examined and studied on a broader base; and results from other spheres of biological research may be applied upon the cancer questions.

Considering the dominating influence of age on the development of cancer, it seems necessary for the future cancer research to direct the closest attention to the metabolism of the aged. Here will be one of the future battlefields against cancer. The British *x*-ray expert, Morley Robert, thinks it essential here to produce a mental and bodily youth in the individuals. The war-cry of the medical profession in its struggle against cancer will be: Keep humanity young!

THE RELATION OF MUSCULAR ACTIVITY TO CARCINOMA[1]

A PRELIMINARY REPORT

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Carcinoma in men, especially retired farmers, has been frequently seen by us in the past few years. These patients, men with large, well developed bodies of the "frontiersman" type, usually gave a history of having retired from the farm in good health, a few years previously, to enjoy a well deserved rest. On the other hand we have often talked with farmers, who for one reason or another still shouldered the responsibilities of the farm, who were well and working hard though sixty, seventy, or even eighty years of age. This observation brought us to consider muscular activity in relation to carcinoma.

There have been no deaths from carcinoma among the Turn Teachers (1) in the past thirty years. Thirty deaths from all causes occurred in this period, and we should expect at least three deaths from carcinoma. The Turn Teachers are of necessity active in a muscular sense. While the list is not large, the exception to the rule is interesting.

We have repeatedly noted that patients, clinically diagnosed as precancerous, improved in a most satisfactory manner on a prescription for increased daily exercise, as rope skipping for women, golf and brisk daily walks for men.

Muscular activity stimulates metabolism and retards the approach of senescence and decay. The tuberculosis problem has been greatly simplified by the recognition of the value of rest, sunshine, wholesome food and fresh air. We believe that the carcinoma problem may also be simplified by the recognition of the value of muscular activity in those periods of life most affected by carcinoma, those periods of life when we are prone to "let down" and check our pace. If muscular activity has an intimate relationship to carcinoma, the value of it will be most apparent in prophylaxsis, the field which to date promises the most in the attack on this malady.

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[1] Read before the Hennepin County Medical Society March 6, 1922.

In a perusal of the literature we are unable to find any direct reference to the relation of muscular activity to carcinoma.[2] Hoffman (2) shows that carcinoma is world wide, is a disease of maturity, affecting both sexes and seriously affecting all civilized nations and races. Observations are quoted and statistics are presented showing that carcinoma is more frequent among the unoccupied, among people of independent means, among the leisured and professional classes, among the overnourished, well housed, well fed, and among those who live in the warmer climates. Carcinoma is less frequent among the people who are actively engaged in a gainful occupation, among the hard working classes, among those who live in large industrial centers, among peoples predominatingly poor and of necessity frugal, living on an alimentation just sufficient for their actual needs, and among those who live in the colder climates where the struggle for existence is more difficult. Reading between the lines one common factor is noted, that of the varying degree of muscular activity. The vast majority of people follow the line of least resistance and those of independent means, the leisured and professional classes, and those who live in the tropics are not compelled to do manual labor as a means of gaining a living and hence are less liable to be active in a muscular sense. Attention must be called to Dublin's (3) conclusion with reference to the experience of the Metropolitan Life Insurance Company for 1914, 1915, 1916, that "the cancer mortality rate at ages where the cancer rate is significant, decreases as we go up the economic scale." To reconcile the statistics presented by Hoffman with those of Dublin one must admit that other factors than the economic status are present.

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[2] It is interesting to us to find that in another part of the world similar ideas have been advanced almost simultaneously. Cherry, of Melbourne, on March 1, 1922, read a paper on A Theory of Cancer (published in Med. Jour. of Australia, April 22, abstracted in J. A. M. A., 1922, lxxix, 245) in which he attributes the recent increase of cancer to deficient muscular activity, overnutrition, and lessened skin activity, as a result of modern living conditions of civilized peoples.

Ewing (4) states that "statistics show that sanitary measures which control infectious diseases exercise no such power over carcinoma, which is somewhat conspicuous by a relative failure to attack the poor, the overworked, the underfed, the savage, but chooses a notable proportion of its victims among the well-to-do, the well nourished, the well protected against infectious diseases, and the indolent."

Ewing has noted also that carcinoma seems to be as widespread as are animal species. The mortality from malignant tumors among pet dogs is reported to be surprisingly high. Forms of carcinoma in horses, cattle, and swine are relatively common, carcinoma of nearly every organ of fowls has been reported, mice and rats are subject to carcinoma and made use of extensively in the laboratory. It is very interesting to note that he too calls attention to the rather sharp fall in carcinoma incidence when one takes up the consideration of carcinoma in wild animals. In this respect one must also consider that wild animals have not been subjected to uniform close study, autopsies have been less frequent, and violent deaths have been more common than among the domesticated animals. McCoy (5) reports autopsy findings of 100,000 rats (95 per cent of which were Mus norvegicus) caught during the San Francisco rat campaign about fifteen years ago. Of these rats 103 were found to have tumors, of which 10 proved to be carcinoma. Thus he found that about one rat for every thousand had a new growth, and only 1 in 10,000 had a carcinoma, the remainder being benign tumors or sarcomata. In addition to the factors incident to the study of carcinoma among wild animals, the necessary muscular activity of the animal in its natural wild state may have a bearing on the relative infrequency of reported carcinoma.

Carcinoma has been observed under certain conditions among artificially bred fish (6). It has been clearly shown that fish kept under artificial conditions are more susceptible to the development of carcinoma than are those living under natural conditions. We believe that the enforced inactivity of the fish in ponds and hatcheries is a potent factor in the development of carcinoma. The dietery factor must also be considered.

The present day labor saving machinery, convenient means of transportation, and ultra-conveniences of modern life all tend to make us less active physically, and it appears to us that the increasing curve of carcinoma closely approximates the inauguration of the Age of Machinery.

The recent researches into the finer biochemical changes attending the metabolism of carcinomatous patients point to the development of carcinoma as an individual biological reaction to chronic irritation, governed possibly by the previous state of metabolism. It would seem that we must look to the biochemist for information as to the exact cause of the reversion of the adult cell to fetal characteristics. The researches of Freund and Kaminer (7), Rosenthal (8), Neuberg (9), Koritschoner and Morgenstern (10), and others are very interesting in this connection.

This opportunity is taken for the presentation of a working hypothesis: That human carcinoma may be the reaction to and the result of chronic irritation of adult epithelial tissues bathed in body fluids altered by certain metabolic products as a result of deficient muscular activity.

In advancing the above hypothesis constructive and destructive criticism is invited, as by intensive cooperative effort sufficient data pro and con will be obtained definitely to prove or disprove our contention.

An investigation in an experimental way of some of the phases of muscular activity and its relation to carcinoma is being conducted which will be reported in the near future. We refer especially to the investigation of the effect of muscular activity on experimental carcinoma in mice. We are also preparing to ascertain the effect on human carcinoma of horse serum obtained after graduated amounts of muscular activity.

In an effort at this time to gain some concrete evidence bearing on the subject, a study of carcinoma in Minnesota for the past three years was undertaken. Access to original certificates of death was obtained through the courtesy of the Bureau of Vital Statistics, Minnesota State Board of Health, there being no compilations available. Among 86,838 records examined 6,351 deaths were ascribed to malignant disease for the years 1918, 1919, and 1920. Sarcoma was named as cause of death in 452 records. There were 3135 deaths from carcinoma among males, and these were used as the basis for the present study. Carcinoma among females was not studied at this time because of the difficulty in estimating the amount of muscular activity relative to occupations of this sex. The deaths were tabulated according to occupation and the occupations divided by us into six groups according to our idea of the muscular activity necessary to that occupation. It is to be regretted that no scientific criterion was available for this classification. Some method as suggested by Waller and DeDecker (11) might be applicable if standardized by sufficient usage. We have tried to use our best judgment in each case. A special report was obtained from the Bureau of Census on the number of persons engaged in each occupation based on the Census of 1920. The deaths in each group for three years were totalled, averaged, the number of persons engaged calculated and the death rate computed on the basis of 100,000 persons engaged to bring each group to a common ground for comparison. The death rates are in a measure not absolutely reliable because of the short period of time under consideration and the present day laxness of the average undertaker in completely filling out certificates of death. We grouped the occupations as follows: Group 1. Occupations involving great muscular activity. In this group are included stonecutters, blacksmiths, boilermakers, moulders and the like. Group 2. Occupations involving moderate amounts of muscular activity, as carpenters, cabinet makers, common laborers, masons, plumbers, and the like. Group 3. Includes occupations involving medium amounts of muscular activity as foremen, millers, brewers, agents, printers, etc. Group 4. Occupations involving small amounts of muscular activity, as the professions, office workers, and the like. Group 5. The farmers, because of the seasonal character of their work in Minnesota, were classed alone. Group 6. This group includes those not actively engaged in any gainful occupation, students, idiots, epileptics, inmates of insane hospitals, and those who have retired from gainful occupations.

Hoffman (12) reports the following statistics:

Death rate per 100,000 males, England-Wales, thirty-five years of age and over

	1890–1892	1900–1902
All males	165.5	210.3
Occupied	146.0	180.0
Not occupied	359.0	503.8

The differences in mortality are very striking and we believe they may be explained on the basis of muscular activity.

In Minnesota for the years 1918, 1919, 1920 we have found the death rate per 100,000 males as follows:

1. Those 21 years of age and over:
   Active (groups 1, 2, 3, 4, 5) 103.5
   b. Inactive (group 6) 510.0
2. Those 45 years of age and over:
   a. Active (groups 1, 2, 3, 4, 5) 285.8
   b. Inactive (group 6) 681.0

The figures for Minnesota are seen to parallel roughly those for England and Wales (see chart 1).

In a consideration of the age class 21 years of age and over we have found the death rate from carcinoma lowest in group 1 (in which the necessary muscular activity is greatest) and highest in group 6 (in which the necessary muscular activity is least). There is a stairstep gradation between the extremes. The ratios between the death rates in the various groups may be thus expressed: I:II:III:IV:V:VI: 1::1:1.7:2.1:2.3:3.6:11.8 (see chart 2, table 1).

To ascertain the ratios in the various age classes we have subdivided the active groups:

A. Those twenty-one to forty-four years of age. In this age class the ratios may be expressed thus: I:II:III:IV:V::1:1.4: 1.5:2.2:2.0 (see table 2, chart 3).

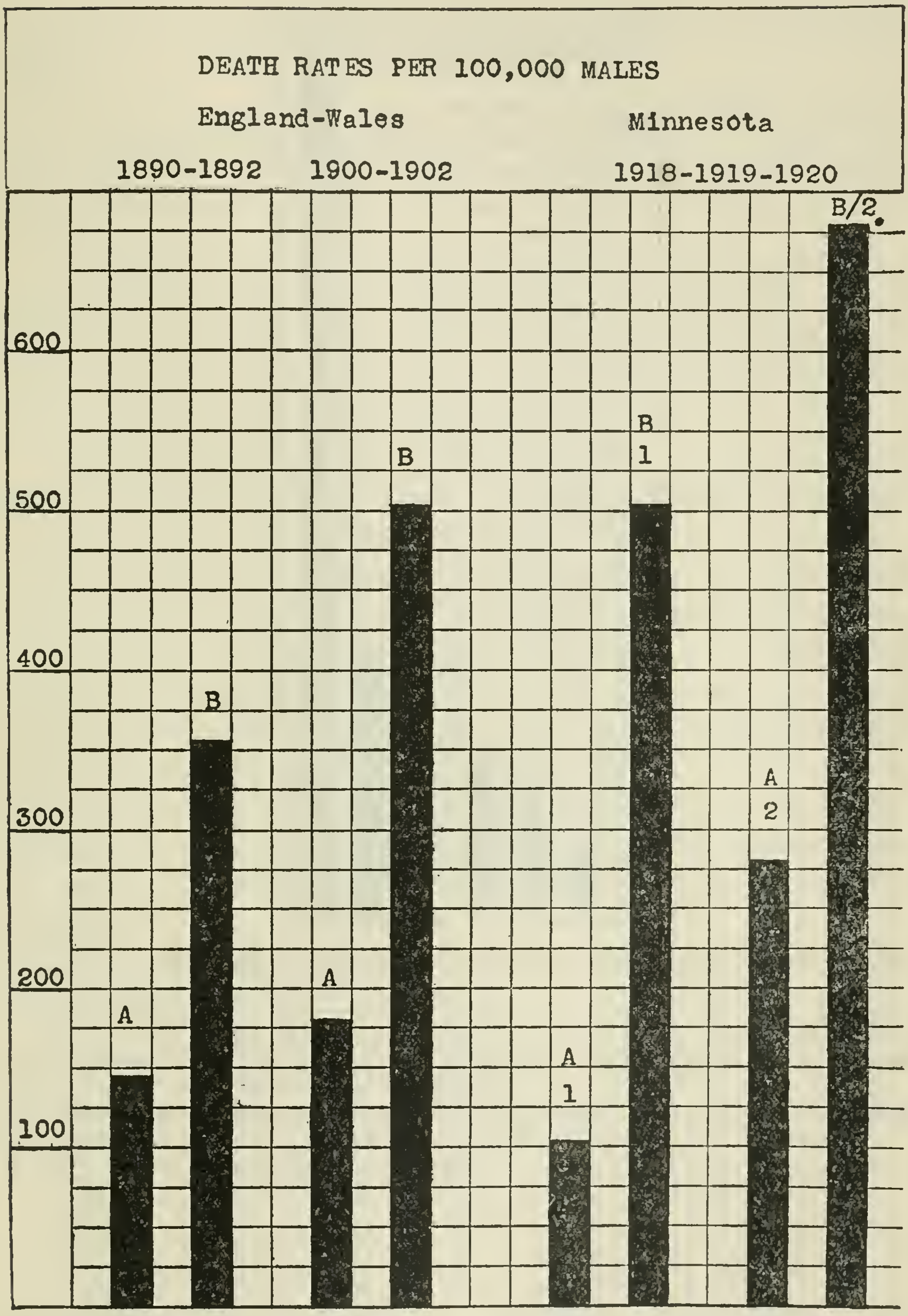

CHART 1. Columns lettered *A* denote occupied; *B*, not occupied; columns not numbered denote those thirty-five years of age and over; numbered *1*, twenty-one years of age and over; *2*, forty-five years of age and over.

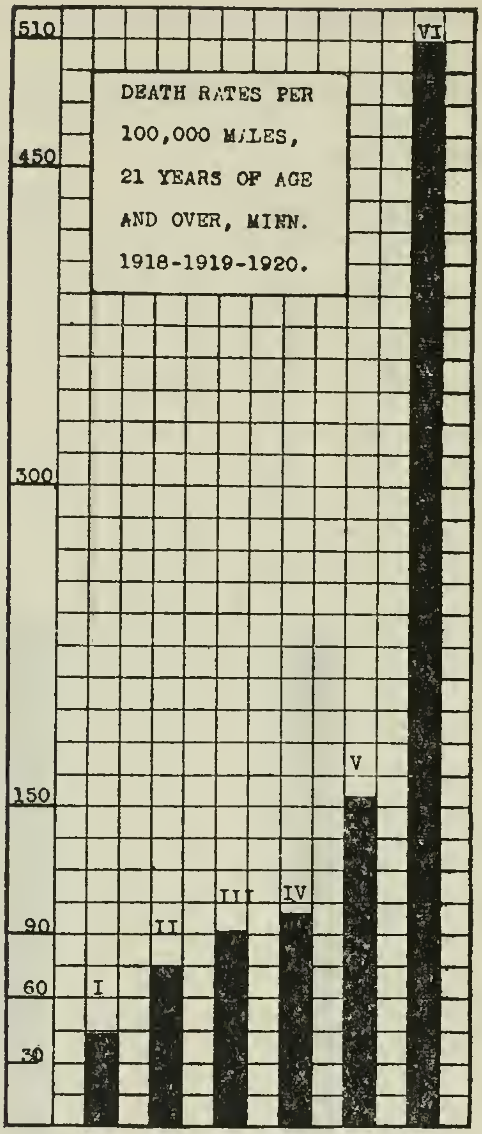

Chart 2. Columns Numbered I, II, etc., Refer to Occupation Groups 1, 2, etc.

TABLE 1

Death rates per 100,000 males, twenty-one years of age and over, Minnesota, 1918, 1919, 1920

GROUP	NUMBER OF PERSONS	DEATHS	DEATH RATE
1	46,736	20.0	43.0
2	168,460	128.0	75.8
3	84,523	77.0	91.1
4	211,824	213.9	100.8
5	170,483	267.7	156.6
1,2,3,4,5	682,026	706.4	103.5
6	64,503	330.0	510.0

B. Those forty-five to sixty-four years of age. The ratios existing are I:II:III:IV:V::1:1.8:2.1:2.0:2.0 (see chart 4, table 3).

C. In those sixty-five years of age and over we have found the difference less striking as might be expected for this age class, the ratios being: I:II:III:IV:V::1:1.5:1.1:1.4:1.9 (see table 4, chart 5).

D. Considering those of another age class, from twenty-one to sixty-four years of age, we find there is progressive increase in death rate with progressive diminution in amount of necessary muscular activity. The ratios may be expressed: I:II:III:IV:V::1:1.6:2.3:2.4:3.1 (see table 5, chart 6).

In all the age classes considered the death rate from carcinoma among those actively engaged in a gainful occupation is roughly inversely proportional to the amount of muscular activity necessary to that occupation. The only out and out exception to the rule is in group 5, the farmers, for which we lack a satisfactory explanation except that the seasonal character of the occupation in Minnesota may have a bearing, in that during the spring and fall seasons the work is very heavy, while in the winter and summer, especially in winter, the work is very light.

The experience of the Industrial Department of the Metropolitan Life Insurance Company (13) discloses that cancer causes the following percentage of total deaths for the following occupations:

Group 1. Blacksmiths 7.6 per cent; miners 4.6; moulders 3.9; average for the group 5.9 per cent.

Group 2. Laborers 5.5; longshoremen 4.3; machinists 4.5; masons 6.5; average 5.2 per cent.

Group 3. Teamsters 3.6; printers 2.7; painters 4.1; track laborers 4.7; average 3.8 per cent.

Group 4. Clerks 3.1; cigarmakers 5.2; average 4.2 per cent.

Group 5. Farmers 7.6 per cent.

The above figures do not indicate any direct influence of the occupational disease factor.

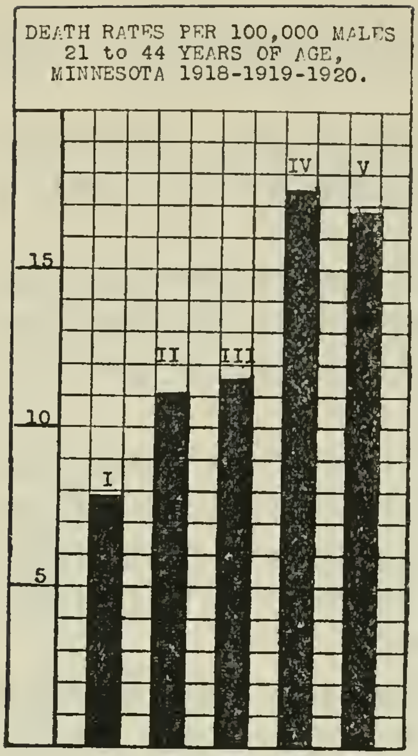

CHART 3

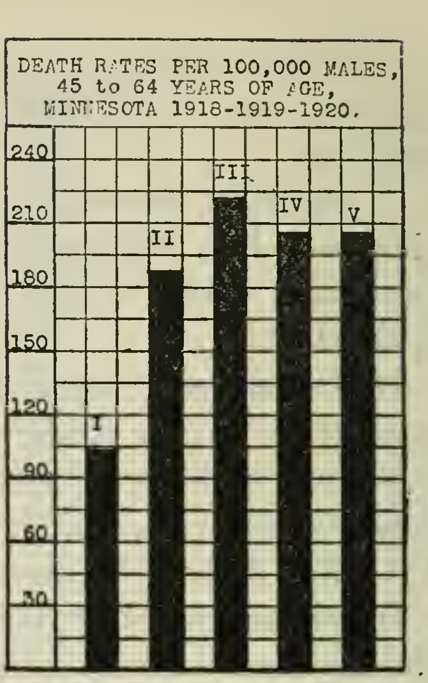

CHART 4

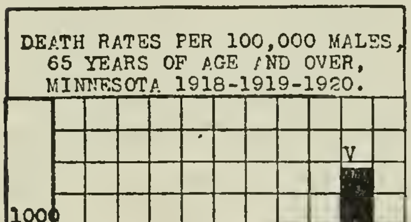

CHART 5

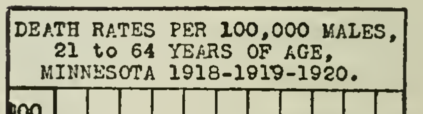

CHART 6

CHART 3. COLUMNS NUMBERED I, II, ETC., REFER TO GROUPS 1, 2, ETC.
CHART 4. COLUMNS NUMBERED I, II, ETC., REFER TO GROUPS 1, 2, ETC.
CHART 5. COLUMNS NUMBERED I, II, ETC., REFER TO GROUPS 1, 2, ETC.
CHART 6. COLUMNS NUMBERED I, II, ETC., REFER TO GROUPS 1, 2, ETC.

TABLE 2

Death rates per 100,000 males engaged, twenty-one to forty-four years of age, Minnesota 1918, 1919, 1920

GROUP	NUMBER OF PERSONS	DEATHS	DEATH RATE
1	34,587	2.7	7.8
2	128,233	14.3	11.1
3	58,362	6.7	11.5
4	141,902	24.7	17.4
5	93,661	15.7	16.7

TABLE 3

Death rates per 100,000 males engaged, forty-five to sixty-four years of age, Minnesota, 1918, 1919, 1920

GROUPS	NUMBER OF PERSONS	DEATHS	DEATH RATE
1	11,057	11.3	102.2
2	4,373	65.3	189.9
3	22,955	51.0	221.1
4	61,321	126.0	205.5
5	66,168	136.0	205.5

TABLE 4

Death rates per 100,000 males engaged, sixty-five years of age and over, Minnesota, 1918, 1919, 1920

GROUPS	NUMBER OF PERSONS	DEATHS	DEATH RATE
1	1,092	6.0	549.4
2	5,854	48.3	825.0
3	3,206	19.3	602.0
4	8,601	65.0	755.7
5	10,654	115.7	1085.0

TABLE 5

Death rates per 100,000 males engaged, twenty-one to sixty-four years of age, Minnesota, 1918, 1919, 1920

GROUPS	NUMBER OF PERSONS	DEATHS	DEATH RATE
1	45,644	14.0	30.6
2	162,606	79.7	49.0
3	81,317	57.7	70.9
4	203,223	148.7	73.1
5	159,829	151.7	94.9

A tabulation of the ages at death in the different occupation groups discloses a rather close approximation of extremes and averages so it appears the differences in death rates shown above can not be wholly explained on a basis of age incidence; that is, there is not sufficient difference between the ages of those in the different occupation groups to explain the ratios found to exist between the death rates in the various groups of occupations (see table 6).

TABLE 6

Ages at death, males, Minnesota, 1918, 1919, 1920

GROUPS	NUMBER OF DEATHS	EXTREMES	AVERAGE AGE AT DEATH
1	67	24–81	57.3
2	379	29–91	60.3
3	201	29–83	57.7
4	667	24–88	56.8
5	878	25–90	62.2
6	984	27–103	70.4

SUMMARY

In a preliminary report attention is called to a new factor in carcinoma etiology. Carcinoma has been frequently observed in retired farmers, seldom in active individuals. Precancerous patients have improved clinically with increased muscular activity. Attention is called to observations of less carcinoma among those of necessity physically active. The reported incidence among domesticated animals is greater than in wild animals. A working hypothesis is advanced: That human carcinoma may be the reaction to and the result of chronic irritation of adult epithelial tissue bathed in body fluids altered by certain metabolic products as a result of deficient muscular activity. From a study of carcinoma deaths among males in Minnesota for three years it appears that the death rate in those who are active is greatly exceeded by the death rate in those who are inactive. From a study of the death rates of those who are actively engaged in a gainful occupation it appears that the death rate is lowest in those occupations involving the greatest amounts of necessary muscular activity, and is highest in those occupations involving the least amounts of muscular activity. The age

incidence factor of the cases studied does not explain the variations shown. The figures from the experience of the Metropolitan Life Insurance Company do not show any marked influence of occupational disease on carcinoma death rate. Additional study and accumulation of data are necessary to establish definitely the status of the relation of muscular activity to carcinoma.

CONCLUSIONS

1. Carcinoma constitutes a serious menace to the adults of all civilized races.
2. It appears that the recent increase has accompanied the advent of the Age of Machinery.
3. The reported incidence among the lower animals appears inversely proportional to the degree of muscular activity necessary to the existence of the animal.
4. The death rate in males actively engaged in a gainful occupation is less than the death rate in those not actively engaged in any gainful occupation.
5. The death rate among males actively engaged in a gainful occupation is inversely proportional to the degree of muscular activity necessary for that occupation.
6. A new factor in carcinoma etiology is proposed.
7. A working hypothesis is suggested.

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